JOURNAL OF AGRICULTURAL RESEARCH

VOL. 42 WASHINGTON, D. C., FEBRUARY 1, 1931 NO. 3

A STATISTICAL STUDY OF THE RELATION BETWEEN VARIOUS EXPRESSIONS OF FERTILITY AND VIGOR IN THE GUINEA PIG [1]

By GEORGE HAINES [2]

Senior Animal Husbandman, Office of Experiment Stations, United States Department of Agriculture

PLAN OF THE STUDY

The primary object of the present study was to determine the relation between various measurable components of fertility and vigor with reference to the interval between the reaction of each to common environmental influences. The relationships were measured by simple correlations between the indices for eight characteristics which were calculated for 217 consecutive months (December, 1906, to December, 1924, inclusive) from the records on the inbred guinea pigs in the colony belonging to the Animal Husbandry Division, Bureau of Animal Industry of the department. Bulletins dealing with the effects of inbreeding by brother and sister matings in the colony have been published by Wright (*16*, *17*) [3] and Wright and Eaton (*18*).

The size of the population studied, together with the distribution by size of litter and sex, is given in Table 1. The litter sizes in this stock varied from 1 to 8, averaging 2.583 ± 0.0041 young per litter. The total number of individuals in each litter size was large except in litters of 7 and 8. It is of interest to note that 82.79 per cent of the entire population was born in litters of 2, 3, and 4. The sex of 391 individuals was not determined, and the birth weights of 243 individuals were not recorded.

THE MONTHLY INDICES

Indices were calculated for each month from December 1906, to December, 1924, inclusive, for litters per 100 matings, average size of litter, sex ratio, average birth weight, average gain between birth and weaning, percentage of young born alive, percentage raised of those born, alive, and number raised per 100 matings.

[1] Received for publication Mar. 21, 1930; issued February, 1931.

[2] The results presented in this paper are taken from a part of a thesis offered to the University of Maryland in partial fulfillment of the requirements for the degree of doctor of philosophy. The data on which the paper is based are the property of the Animal Husbandry Division, U. S. Department of Agriculture, of which E. W. Sheets is chief, and the author wishes to express his sincere appreciation to the division for permission to make this analysis. He desires also to acknowledge his gratitude to Sewall Wright, collaborator of the division, for suggesting, directing, and commenting on the study, as well as to De Voe Meade, of the University of Maryland, who jointly supervised the work, and to H. C. McPhee and O. N. Eaton, of the Animal Husbandry Division, for assistance. Acknowledgment is also made for the work of Helena J. Haines, who calculated the correlation coefficients and checked many of the computations.

[3] Reference is made by number (italic) to Literature Cited, p. 163.

The necessary steps and means of calculating each index, which also serve as definitions, were as follows:

1. Litters per 100 matings. The number of litters per 100 matings was the index indicating the frequency at which litters occurred and was calculated as one hundred times the number of litters born in a particular month divided by the number of pairs mated in that month. As nearly all matings were made between brothers and sisters at 33 days of age, when the males, at least, and usually both males and females, were sexually immature, a new mating was not counted until two months after it occurred. The basis for the correction was Wright's (*15*) findings that the minimum age at which males may sire litters is about 60 days, and the average age at which first litters are born is 5.9 months. Mature animals require some time to become acquainted in new matings. Matings were stopped in the month after that in which the female died and two months after the male died. The final value was corrected to a 30-day month.

2. Size of litter. The average size of litters was readily calculated from the total number of litters of all sizes and the number of individuals born in a particular month.

3. Sex ratio. Sex ratio was expressed as percentage of males rather than by the more customary means—the number of males per 100 females.

4. Birth weights and gains. Weights show a great deal of variation, which is in part related to the size of litter, mortality, and sex. The birth weights were calculated separately for each litter size, for each sex, and for those born dead, those dying between birth and weaning, and for those raised to weaning; but since gains up to weaning were likewise to be obtained, it was decided to use the average weights of those which were raised to weaning as the monthly index.

In calculating the gains, the average weaning weight (at 33 days) for each month, corrected for size of litter and sex, was first determined and the difference between the corrected birth weight and the corrected 33-day weight gave the gain for the month. The gains for a particular month referred to the calendar month in which the animal was born rather than to the month in which the gain was made; thus, the relation between birth weights and gains is not lost.

The basis for the corrections of the birth weights and gains to eliminate the effect of size of litter and sex is described later.

5. Percentage of young born alive and percentage raised. The percentage of young born alive and the percentage raised of those that were born alive are also affected by the size of litter, and a method for the elimination of this effect is described later. The months apply to the month of birth for the percentage raised, as in the case of gains.

6. Young raised per 100 matings. The young raised per 100 matings gives the relation between the actual number of young born in a particular month which were raised to weaning age and the number of pairs of parents mated in the same month. The month referred to is the month in which the individuals were born. This index was corrected to a 30-day month.

EFFECT OF LITTER SIZE AND SEX ON WEIGHTS, GAINS, AND MORTALITY

Various investigators have found that the size of litter and the sex of young have a distinct influence on birth weights, gains to weaning, and mortality. The proportion of large litters also differs in different seasons. It was therefore necessary in studying the influence of season on these characteristics to determine first the influence of litter size and sex.

Because of the small numbers involved and their exceptional relations to the other litter sizes, litters of 7 and 8 were eliminated from the study of seasonal influences, but to make the data complete they are usually included in the tables giving averages for litter size. In the total population (Table 1) litters of 8 occurred at the rate of only 1 to nearly 4,000 litters, and litters of 7 occurred at the rate of 1 to each

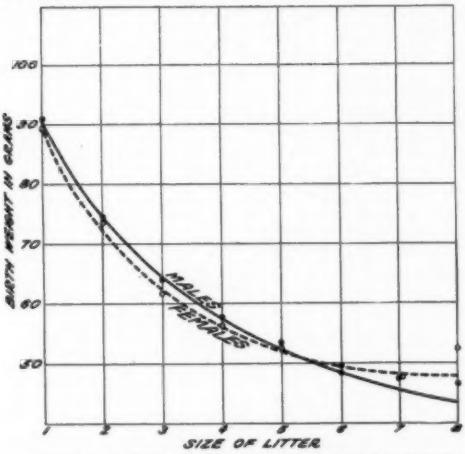

FIGURE 1.—Average birth weights of males and of females born dead in each litter size from litters of 1–6, fitted by second-order parabola, litters of 7 and 8 fitted by extrapolation. For males $y^{-1}=0.008571+0.002592x-0.000099x^2$; for females $y^{-1}=0.008083+0.003255x-0.000207x^2$. Solid lines show birth weights of males and broken lines birth weights of females. Solid dots are observed values for males and circles observed values for females

629 litters. The large litters also came in the most favorable seasons. Their omission from the calculations of the monthly indices could have no significant influence on the final results.

TABLE 1.—*Number of litters and individuals included in the animals studied*

Size of litter	Litters	Individuals	Born alive	Raised to weaning	Males [a]	Females [a]
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
1	2,037	2,037	1,650	1,351	1,008	1,001
2	3,952	7,904	6,854	5,759	3,970	3,854
3	3,693	11,079	9,442	7,892	5,538	5,405
4	1,641	6,564	5,074	4,070	3,261	3,209
5	484	2,420	1,710	1,333	1,202	1,179
6	116	696	431	303	353	332
7	19	133	79	52	67	63
8	3	24	17	15	15	9
Total of 1–6	11,923	30,700	25,161	20,708	15,332	14,980
Total of all	11,945	30,857	25,257	20,775	15,414	15,052

[a] Sex was not recorded for 391 individuals.

BIRTH WEIGHTS

Wright (*16*, *17*) pointed out that the birth weights of guinea pigs vary greatly, ranging from approximately 40 to 150 gm. for those that are raised. He also stated that size of litter was at least a very important contributory factor to the variability in weights, and found a correlation of -0.658 ± 0.007 between size of litter and mean birth weights of litter mates (*15*). Table 2, giving the average birth weights of the individuals in each of the three groups of males and females, according to size of litter, shows a definite relation of birth weight to size of litter. Notwithstanding the extreme variability of individuals, Figures 1 and 2 show that the average birth weights of those born dead, those dying between birth and weaning, and

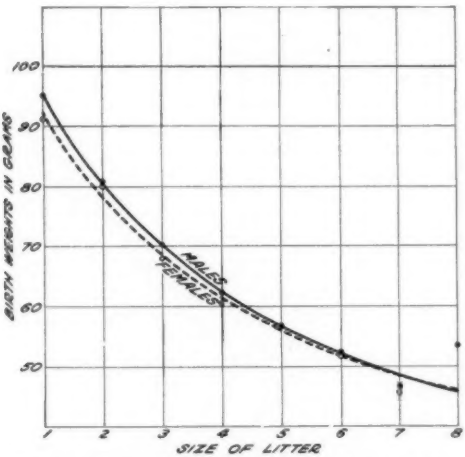

FIGURE 2.—Average birth weights of males and females dying between birth and weaning in each litter size fitted from litters of 1–6 by second-order parabola, litters of 7 and 8 fitted by extrapolation. For males $y^{-1}=0.008449+0.002088x^2-0.000051x^2$; for females $y^{-1}=0.008758+0.00214\,^2x-0.000065x^2$. Solid line shows birth weights of males and broken line birth weights of females. Solid dots are observed values for males and circles observed values for females

those raised to weaning of each sex form relatively smooth and regular curves when plotted according to size of litter.

In all six groups the average birth weights of litters of 1 are decidedly the largest. A sharp downward trend in birth weights follows for litters of 2 and 3, but after this the weights decrease at a much slower rate. Litters of 7 and 8 show considerable irregularity, probably due to the small numbers.

The mortality of the newborn young in each litter size appears to be closely associated with the average birth weight. In litters of 1 the males raised weighed at birth nearly 13 gm. and the females 14 gm. more than those which died between birth and weaning. A difference of 8 to 10 gm. is maintained throughout the different litter sizes in both sexes with small fluctuations, except in litters of 8. There is a smaller but quite consistent difference in the birth weights of those which were born alive but died before weaning and those

which were born dead. The significance of the differences between the birth weights of the different groups was tested for males and females according to Student's method as described by Fisher, using litters of 1 to 6, inclusive (*1*, *2*). Table 3 gives the data needed for interpretation.

TABLE 2.—*Average weights of guinea pigs according to size of litter*

MALES

Size of litter	Birth weight of animals born dead	Birth weight of animals that died	Birth weight of animals raised to weaning	Weaning weight	Gain in weight to weaning	
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Per cent of birth weight*
1	90.95	95.06	107.75	280.18	172.43	160.03
2	74.68	80.98	91.61	251.17	159.56	174.17
3	64.04	70.15	79.39	221.23	141.84	178.66
4	57.58	62.32	71.67	204.12	132.45	184.81
5	53.28	56.84	66.99	198.87	131.88	196.87
6	48.28	52.26	62.50	192.56	130.06	208.10
7	47.54	46.71	55.83	201.38	145.55	260.70
8	46.50	53.50	55.80	145.40	89.60	260.57
Average of 1–6	63.85	71.10	82.09	228.22	146.13	178.01
Average of all	63.65	70.93	82.02	228.12	146.10	178.13

FEMALES

1	89.41	91.36	105.70	273.79	168.09	159.03
2	73.60	79.97	89.42	241.26	151.84	169.81
3	61.84	67.93	77.60	215.40	137.80	177.58
4	56.22	60.80	69.02	196.64	127.62	184.90
5	52.38	56.51	64.93	190.02	125.09	192.65
6	49.51	51.82	59.80	179.30	119.50	199.83
7	47.68	45.85	55.50	178.71	123.21	222.00
8	52.33		64.50	141.00	76.50	118.60
Average of 1–6	62.78	69.42	79.92	220.48	140.56	175.88
Average of all	62.64	69.28	79.85	220.34	140.49	175.94

TABLE 3.—*Comparison of birth weights of guinea pigs in groups according to fate*

Groups compared	Mean differences	Standard deviation [a] of differences	*t*
	Grams	*Grams*	
Males born dead and those that died	4.80	1.154	10.19
Females born dead and those that died	4.24	1.847	5.63
Males died and males raised	10.38	1.252	20.31
Females died and females raised	9.68	2.382	9.96

[a] $\sigma = \sqrt{\frac{\Sigma d^2}{n-1}}$

With six litter sizes there are five degrees of freedom, and from Fisher's table of *t* (*2*), if $t = 4.032$, P is 0.01. Since all values of *t* observed exceed 4.032, there is less than 1 chance to 99 that the differences are not significant. It must, therefore, be concluded that the average birth weights of the different groups differ significantly in both sexes.

Slight differences in the shape of the curves for the different groups are apparent in both sexes, but the close approach to parallelism in the two sexes, with the males slightly heavier in all groups and in nearly all litter sizes, is beyond expectation. This is best noted in Figures 1, 2, and 3. Because of the uniformity of the differences within different litter sizes, males would appear to be heavier than females at birth, but the average difference between males and females in litters of 1–6 for those born dead was 0.98 gm., and for those dying between birth and weaning, 1.54 gm. The respective standard deviations of the differences were 1.170 and 1.272 gm., respectively, from which the values of t were calculated as 2.05 and 2.96. The corresponding P values were 0.1 and 0.04. Thus the sex difference in the birth weights of those born dead is not significant, and for those

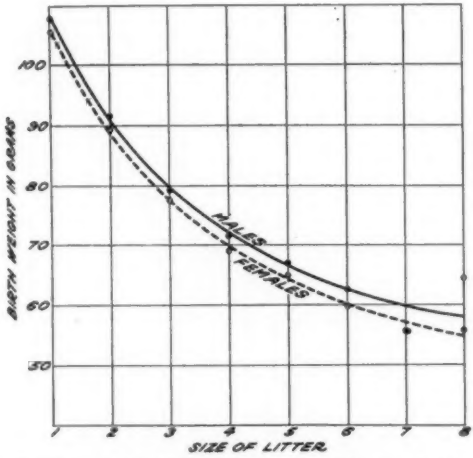

FIGURE 3.—Average birth weights of males and females raised to weaning in each litter size fitted from litters 1–6 by second-order parabola, litters of 7 to 8 fitted by extrapolation. For males $y^{-1}=0.007303+0.002050x-0.000101x^2$, for females $y^{-1}=0.007441+0.002089x-0.000092x^2$. Solid line shows birth weights of males and broken line birth weights of females. Solid dots are observed values for males and circles observed values for females

that died it can not be considered certain. The birth weights of males raised are also greater than the birth weights of females raised; the mean difference in litters of 1–6 was 2.24 gm. with σ of 0.361 gm. making t 15.20 and P much less than 0.01. With five degrees of freedom, when t is 4.032, $P=0.01$.

It may be concluded that birth weights are significantly related to size of litter and to mortality of young before and after birth, and that the birth weights of those individuals which are raised, as well as those which die between birth and weaning, are related to sex.

WEANING WEIGHTS AND GAINS

The effects of sex and size of litters on weaning weights and gains from birth to weaning are brought out in Table 2. Figure 4 shows a pronounced effect of sex on weaning weight similar to that on birth weight. Males are heavier in all litter sizes. The relation of size of

litter to weaning weight is also similar to the relation of size of litter to birth weight, especially in the small litter sizes.

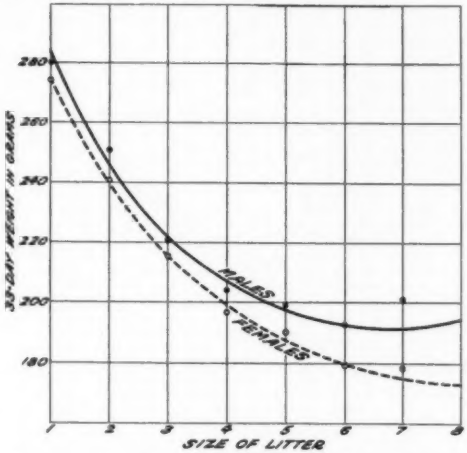

FIGURE 4.—Average weights of males and females at 33 days of age (weaning) born in each litter size fitted, from litters of 1–6, by second-order parabola, litters of 7 and 8 fitted by extrapolation. For males, $y^{-4}=0.0028884+0.0006922x-0.0000514x^2$; for females, $y^{-4}=0.0030235+0.0006550x-0.0000388x^2$. Solid line shows weaning weights of males, and broken line weaning weights of females. Solid dots are observed values for males and circles observed values for females

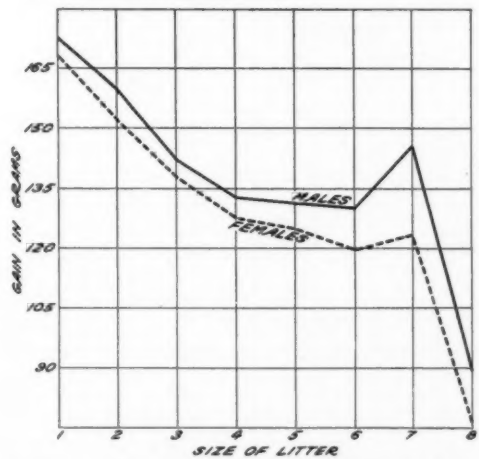

FIGURE 5.—Average gains between birth and weaning (33 days) for males and females born in different-sized litters. Solid line shows gains of males and broken line gains of females

The mean difference between the weaning weights of males and females in litters of 1–6 was 8.62 gm. and the standard deviation of the difference was 2.731 gm.; t was, therefore, 7.73, and with five degrees of freedom, P is considerably less than 0.01.

The weaning weight is a combination of the birth weight and the gain made between birth and weaning. Figure 5 shows the influence of sex and litter size on the gains between birth and weaning. Al-

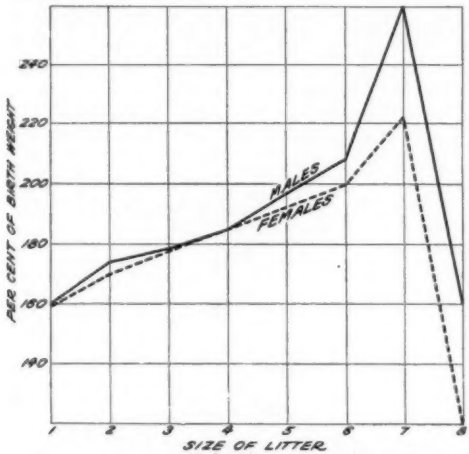

FIGURE 6.—Gain between birth and weaning (33 days) of males and females born in the different-sized litters, expressed as percentage of birth weight. Solid line shows percentage gain for males and broken line percentage gain for females

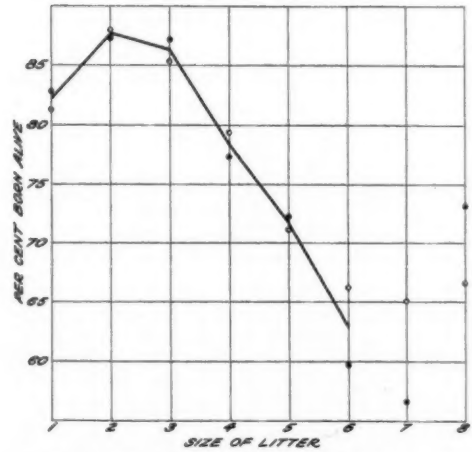

FIGURE 7.—Percentage of males and females born alive in the different litter sizes. Solid line shows the mean percentage for the two sexes in litters of 1–6, solid dots the percentage of males born alive, and circles the percentage of females born alive in each litter size

though it might be assumed that in an animal born as mature as the guinea pig and which is so little dependent on its dam's milk supply (*5*), the size of litter would have practically no effect on postnatal

growth, it is very apparent that, whether due to the care and food supplied by the dam or to their larger birth weights, the young born in the small litters have a distinct advantage in the amount of gain. The percentage increases in weight during the weaning period (Table 2 and fig. 6) are conversely related to birth weights. The increase for males in litters of 6 was calculated at 208.10 per cent, but in litters of 1 it was 160.03 per cent, with comparable values for females. Thus an increased relative gain accompanies an increase in litter size and tends somewhat to offset the percentage differences between the weights at birth in the different sized litters. Considering the

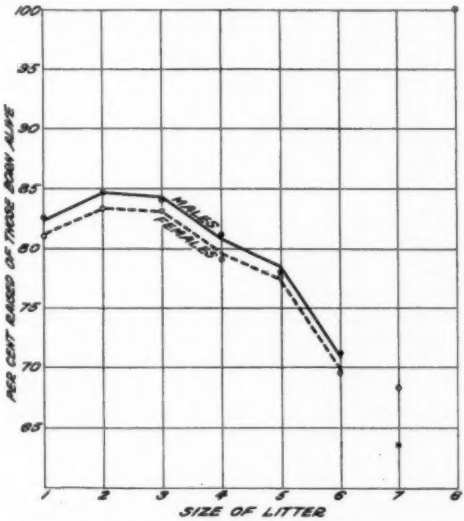

FIGURE 8.—Percentage of males and females born alive which were raised to weaning in each litter size. Solid line shows the percentage of males and the broken line the percentage of females in each litter size from 1–6 as calculated from the mean of the two sexes by the method of weighing explained in the text. Solid dots give observed percentages for males and circles observed percentages for females in each litter size

small numbers involved, litters of 8 can hardly be regarded as a reliable exception.

The mean difference between the gains of males and females in litters of 1–6 was 6.38 gm. with a standard deviation of 2.509 gm., giving a value for t of 6.03; with five degrees of freedom, P is therefore less than 0.01.

The weaning weights and gains between birth and weaning of males were thus significantly greater than those of females.

MORTALITY OF YOUNG

A study of Table 4, which gives the number of animals born dead, the number that died, and the number raised of each sex in litters of different sizes, shows that size of litter bears an important relationship to mortality. The percentage of young born alive is shown in Figure 7, and the percentage raised of those that were born alive is shown in Figure 8.

TABLE 4.—*Number and mortality of males and females born in each litter size*

Size of litter	Males					Females				
	Born dead	Died	Raised to weaning	Born alive	Raised to weaning of young born alive	Born dead	Died	Raised to weaning	Born alive	Raised to weaning of young born alive
	Number	*Number*	*Number*	*Per cent*	*Per cent*	*Number*	*Number*	*Number*	*Per cent*	*Per cent*
1	173	145	690	82.84	82.63	186	154	661	81.42	81.10
2	503	533	2,934	87.33	84.63	467	562	2,825	87.88	83.41
3	715	772	4,051	87.09	83.99	786	778	3,841	85.46	83.16
4	737	473	2,051	77.40	81.26	659	531	2,019	79.46	79.18
5	332	190	680	72.38	78.16	339	187	653	71.25	77.74
6	142	61	150	59.77	71.09	112	67	153	66.27	69.55
7	29	14	24	56.72	63.16	22	13	28	65.08	68.29
8	4	2	9	73.33	81.82	3	.....	6	66.67	100.00
Total of 1–6	2,602	2,174	10,556	*83.03	*82.92	2,549	2,279	10,152	*82.98	*81.67
Total of all	2,635	2,190	10,589	*82.91	*82.86	2,574	2,292	10,186	*82.90	*81.63

* Weighted average.

The one very striking feature of these data is that the chances of survival for individuals born in litters of 1 are less than for those born in litters of 2 and 3. It has been observed by Wright (*16*, *17*) that frequently very large animals are born dead, evidently due to difficulties at parturition. Such difficulties might have had some influence on the smaller percentage born alive in litters of 1, but would in no way explain the lower percentage raised of those born alive in this litter size. The explanation for the higher mortality in litters of 1 is probably the lowered vigor of dams producing litters as small as 1.

The chances of the animals being born alive in litters of 4, 5, and 6 decrease very rapidly with each increase in litter size. The fact that the curve for the percentage raised of those born alive does not drop off nearly so rapidly with larger litters as the curve for percentage born alive indicates the lesser dependence on the dam and is, no doubt, also a result of the reduced size of the larger litters through heavier mortality at birth.

It is very apparent that the difference in mortality between the sexes is less than the difference in weights. The percentages of the two sexes born alive alternate at each litter size, indicating that there is no significant difference between males and females in this respect; the weighted averages were 83.03 per cent for males and 82.98 per cent for females in litters of 1–6.

There is a slight but constantly higher percentage of males than of females raised in litters of 1–6 (fig. 8), the mean weighted averages being 82.92 per cent for males and 81.67 per cent for females. The percentages raised in the different litter sizes indicated a sex difference in this characteristic.

To test the likelihood of the distribution of males and females within normal expectation, χ^2 was determined for fourfold tables for each litter size from 1–6, according to the method outlined by Fisher (*2*).

The values of χ^2 and P are given in Table 5. The χ^2 values in litters of 3 and 4 indicate the distributions of the sexes among the young born dead and those born alive which would occur less than 5

times in 100 trials if there were equal mortality of the two sexes. There were more males than females born alive in litters of 3 (87.09 per cent males and 85.46 per cent females), and more females than males born alive in litters of 4 (79.46 per cent females and 77.40 per cent males). The alternation of the two sexes in these and the other litter sizes, indicate that there was no difference in the mortality of the two sexes at birth, even though the deviation from equality in the litter sizes would be expected to occur but about twice in 100 times. None of the χ^2 values for the animals that died and those that were raised are without the normal expectation.

TABLE 5.—*Chi-square test of the distribution of the sexes according to fate*

Size of litter	Born dead and born alive		Died and raised to weaning	
	χ^2	P	χ^2	P
1	0.689	0.30–0.50	0.651	0.30–0.50
2	.550	.30–.50	1.898	.10–.20
3	6.150	.01–.02	1.204	.20–.30
4	4.074	.02–.05	3.469	.05–.10
5	.377	.50–.70	.045	.80–.90
6	3.090	.05–.10	.123	.70–.80
Total N=6	14.930	.02–.05	7.390	.20–.30

Since males uniformly excel females in the percentages raised in litters of 1–6, Student's method (*1*, *2*) affords a test for the significance of the difference. Comparison was, therefore, made of the percentage of males and of females raised in litters of 1–6. The mean difference in the percentage raised was 1.27 per cent in favor of the males, with a standard deviation of 0.586 per cent. Thus t was 5.31, for which P with five degrees of freedom is less than 0.01. This difference is, therefore, clearly significant.

In litters of 1–6 the percentages of males in the different groups were as follows: In the group born dead, 50.145 ± 0.470 per cent,[4] died 48.821 ± 0.505 per cent, and raised 50.976 ± 0.2641 per cent. The difference between the percentage of males among those born dead and died was 1.6935 ± 0.6901 per cent, and between those died and raised 2.1545 ± 0.5568 per cent.

The data presented show that the size of litter in which young are born bears an important relation to mortality at birth and between birth and weaning, litters of 2 and 3 being most favorable for survival. Sex had no influence on mortality at birth, but between birth and weaning the mortality of females was slightly greater than that of males.

SEX RATIO

Among all young born there were 50.5941 ± 0.1929 per cent males, a probably significant departure from 50 per cent. The percentages of males in litters of 1, 2, 3, 4, 5, 6, 7, and 8 were 50.17, 50.74, 50.61, 50.42, 50.48, 51.53, 51.54, and 62.50 per cent, respectively. The χ^2 test for the departure of the sex ratio in the different litter sizes (1–8) from the mean ratio does not give support to the significance of the departures. χ^2 was 2.0056. With $n=7$, P is 0.96. Departures as

[4] Probable error for percentage of males equals $0.6745\sqrt{\frac{\text{Per cent males} \times \text{per cent females}}{n}}$

great as these from the mean are thus to be expected 96 times in 100 trials.

The significance of the deviations from equality of the sexes was tested by Fisher's t method, using only litters of 1–6 in which the numbers were relatively large. The mean difference was 1.317 per cent, with a standard error of ± 0.3823, making t, 3.44—a value to be expected but about twice in 100 trials if there were no difference. If litters of 7 and 8 were included, the probability would be less.

DISCUSSION

Many authors have called attention to the effects of litter size on birth weights, including Gates (*3*) and Parkes (*13*), in mice, Hanson and Heys (*4*), in the rat; Kopéc (*6*, *7*), in the rabbit; and Prawocheński and Kaczkowski (*14*), in sheep and other animals. Minot (*11*) made a study of the growth of guinea pigs from birth to maturity. He found that the size of litter had an important influence on birth weights, especially in the smaller litters. The differences in the birth weights he attributed mainly to differences in the length of gestation periods, but Wright (*15*) found that size of litter was much more effective in reducing birth weights by reducing the rate of growth than by causing early parturition. Differences in location or relation to other fetuses in the uterus may also influence the differences in the size of individuals in the same litter. Marshall (*10*) has discussed the possible effects of limited nutrition of the dam, mainly with negative conclusions. When the animals are more mature at parturition and the numbers of young are relatively small, the effect of additional numbers seems particularly important.

In the present study the birth weights, weaning weights, and gains of the two sexes conformed with the general findings of others in almost all classes of animals, males being generally heavier.

It is more difficult to find an explanation of the effect of size of litter on mortality and especially on prenatal mortality. The operation of lethal factors would be equally important in all litter sizes and, therefore, can not be considered. Nutrition might be a limiting factor, provided the young became very much weakened from lack of food, which is quite likely to have been a factor, as is indicated by the similarity between the birth weights of those dying between birth and weaning and those dying at birth as contrasted with the considerably greater average weight of those raised, but the condition and age of the dam and maturity of the young seem to be more likely possibilities. Here again the dam's influence particularly seems to be somewhat complicated. How can a female in sufficiently good condition to produce a litter of 5, 6, or 7 be considered in too poor condition to raise as large a proportion of her young as a female producing a litter of 2 or 3? The explanation is possibly related to the maturity of the young at birth. Because of the greater mortality at birth in large litters the actual number which the dam has to care for is not very much greater than the number cared for in smaller litters. Maturity alone, however, can not be the whole story, for it is well known and has been pointed out by Minot (*11*) and Wright (*16*, *17*) that litters of 1 are most mature at birth, and yet both the percentages that are born dead and those that die between birth and weaning are larger in litters of 1 than in litters of 2 and 3. It may be that the explanation of the effect of litter size on mortality is a combination

of the maturity and competition of the young and the condition and age of the dam. The influence of the dam may apply particularly to litters of 1, whereas the maturity of the young is perhaps more important in large litters.

The sex ratios combined with mortality do not indicate large inequalities in the mortality of the two sexes at the different stages of development. The small differences during the weaning period have been pointed out. Much has been published on the sex ratios of animals, in many cases showing sex differences in mortality, the immature male usually being less vigorous. In the present study, however, females if anything were less viable during the period between birth and weaning.

ELIMINATION OF THE EFFECT OF LITTER SIZE ON WEIGHTS

The primary purpose of determining the average weights of males and females born in litters of different sizes was to get at some means of eliminating such effects in the calculation of monthly indices. The averages when plotted, especially in litters of from 1 to 6, formed relatively smooth curves. A very close fit to practically all the observations for birth weights and the 33-day weights was obtained for males and females in the different groups by fitting to the formula $y^{-1}=a+bx+cx^2$, in which y^{-1} represents the reciprocal of the weight in any litter size, and x in litters from 1 to 6; and a, b, and c are constants. These values for males and females in the different groups are given in Table 6. The fitted curves for each group are shown in Figures 1, 2, 3, and 4. The solid line in each case represents the calculated curve for the weights of the males and the broken line the same for the females. Each observed average value is given as a solid dot for males and a circle for females.

TABLE 6.—*Calculated values of constants in parabola*[a] $y^{-1}=a+bx+cx^2$

Group	a	b	c
Birth weight of males born dead	0.008571	0.002592	−0.000099
Birth weight of females born dead	.008083	.003255	−.000207
Birth weight of males that died	.008449	.002088	−.000051
Birth weight of females that died	.008758	.002142	−.000065
Birth weight of females raised	.007303	.002050	−.000101
Birth weight of females raised	.007441	.002089	−.000092
Weaning weight of males	.0028884	.0006922	−.0000514
Weaning weight of females	.0030235	.0006550	−.0000388

[a] y is weight, x is size of litter and a, b, and c are constants.

Correction factors for the weights of individuals born in any litter size were then calculated for each group from the formula $C_x=\frac{M}{y}$, where C_x represents the correction factor for litter size, x; M is the mean weight of the group including all-sized litters; and y is the calculated average weight of the individuals born in a certain litter size, x. The reciprocals of the calculated weights for each litter size (y^{-1}) were multiplied by the mean of the group to obtain the weight-correction factors given in Table 7.

The correction factors were calculated from the averages for litters of 1–6. In all groups the curves have a tendency to turn up

at or near litters of 7 or 8, thus showing heavier weights for the individuals in larger litters. This is not at all in conformity with the general tendencies observed where the numbers are sufficient, though in certain cases the curves appear to fit the observed data more closely because of turning up. Litters of 7 and 8 were not included in the calculation of monthly indices for weight and mortality.

TABLE 7.—*Correction factors for size of litter*

Item	Correction factor when size of litter is—						Average used as standard
	1	2	3	4	5	6	
							Grams
Birth weight of males born dead	0.7065	0.8530	0.9869	1.1082	1.2168	1.3128	63.8537
Birth weight of females born dead	.6988	.8642	1.0036	1.1169	1.2043	1.2657	62.7810
Birth weight of males that died	.7455	.8831	1.0134	1.1365	1.2523	1.3609	71.0971
Birth weight of females that died	.7521	.8873	1.0134	1.1305	1.2386	1.3377	69.4177
Birth weight of males raised	.7595	.9029	1.0298	1.1400	1.2337	1.3108	82.0935
Birth weight of females raised	.7543	.8992	1.0294	1.1449	1.2456	1.3317	79.9204
Weaning weight of males	.8054	.9282	1.0276	1.1035	1.1559	1.1849	228.2170
Weaning weight of females	.8025	.9212	1.0228	1.1074	1.1748	1.2251	220.4750
							Per cent
Per cent born alive	1.0107	.9476	.9621	1.0585	1.1558	1.3193	83.01
Per cent raised of those born alive	1.0051	.9795	.9847	1.0261	1.0558	1.1767	82.30

ELIMINATION OF THE EFFECT OF LITTER SIZE ON MORTALITY

The correction factors for mortality were not so easily smoothed as those for the weights. After a number of different methods and formulas had been tried, the most satisfactory method seemed to be to strike the mid-point between the values determined for males and females. The reciprocals for the combined percentages of males and females born alive, multiplied by the average for all-sized litters of 1–6, were used as the correction factors for percentage born alive. As no difference between the sexes was established, a single correction value was used for each litter size.

The basis for the correction factors for the effect of litter size on percentage raised was selected as the mid-point between the percentage of males raised and the percentage of females raised, since there were nearly equal proportions of the two sexes among those raised. These points were then modified by the average of one-half the weighted difference between the males and females at each point, and thus parallel curves were constructed. This procedure was carried out only for litters of 1–6 and appeared quite satisfactory.

The mortality correction factors based on the reciprocals of the smoothed values multiplied by the average for litters of 1–6 are also given in Table 7 for the percentage of males and females born alive and raised.

SEASONAL FLUCTUATIONS IN MONTHLY INDICES

All data were tabulated by months, litter size, mortality, and sex. After necessary corrections had been made for size of litter and sex, the means for weight and mortality were used as the indices for each month.

The monthly indices so calculated are presented in Figure 9. The values for the indices are given in the left-hand margin of the figure,

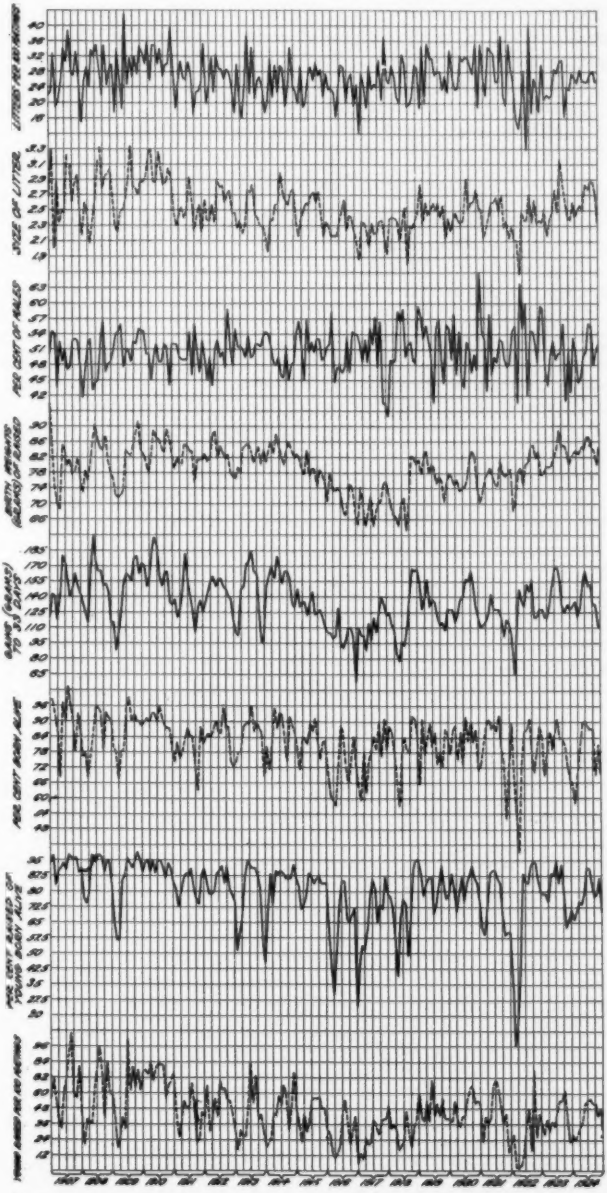

FIGURE 9.—Monthly indices calculated as explained in the text for litters per 100 matings, size of litter, percentage of males, birth weights of those raised to weaning, gains to weaning, percentage born alive, percentage raised of those born alive, and young raised per 100 matings. The vertical lines represent 3-month intervals

while the vertical lines represent the months from December, 1906, to December, 1924, inclusive, in 3-month intervals. In considering any of the series of data the fact that the vigor of the stock was undergoing a decline throughout the period should not be overlooked (Wright (*16*, *17*)). It is also of general interest in this connection that conditions were very favorable in 1910 and rather unfavorable for a period from about 1915 to 1918.

LITTERS PER 100 MATINGS

The division of the time into calendar months proved rather inappropriate for the calculation of this index, as it gave a graph of a rather distinct zigzag or saw-tooth character, resulting from the fact that females producing litters in one month can not produce litters again in the next month because of the 68-day gestation period. Evidently when conditions become favorable the reaction is pronounced and litters are produced by a large portion of the females, which automatically prevents litters from being born to these individuals in the next month. The high and low points of the different years show some tendency toward seasonal effects, but there is much irregularity. There appears to be some tendency for the period from May to August to be a little more favorable with relatively high points in the fall in many years. It is a striking fact that the really high points in the late fall or early winter appear to be related to years in which no high point was observed in a spring or summer month.

It is evident that this index was not entirely satisfactory as a measure of the frequency at which litters were produced. Frequency of litters, being obviously a much-delayed evidence of conditions during a period of over two months prior to the time of parturition, tends further to complicate its expression.

SIZE OF LITTER

As in the case of frequency of litter, size of litter is mainly determined at conception, 68 days before the litter is born. There are, however, influences acting throughout pregnancy which may tend to reduce the size of the litter. In the case of this index there is apparently some cyclic behavior related to the season. From January to April the litter size is generally small, while from June to November it is usually larger. A few years like 1917, 1918, and 1919, however, show considerable irregularity and do not bear this out well.

SEX RATIO

The percentage of males shows much seasonal variation, but even with the irregular character of the graph there appears to be a slight tendency for the ends of the years to be high with a drop during the spring and summer months. The exceptions to this are so frequent, as in 1918, that this index can not be considered as following the season closely. Many years indicate that these variations are related to the behavior of the other indices. For instance, in 1909 practically all the other indices show a relatively high point in January with a drop in March and a rise to July, while the percentage of males goes in the opposite way. Again in 1920, 1921, and 1922 a similar relation is striking, but other cases do not bear this out.

In a test of the goodness of fit of the sex ratio in the individual months to the monthly average sex ratio observed, 50.58 per cent

males, χ^2 was found to equal 173.65. χ^2 tables do not go as high as $n=216$ (217 months were used), but Fisher (*2*) suggests the use of $\sqrt{2\chi}-\sqrt{2n-1}$, in which differences significantly greater than 2 indicate that the data do not conform to expectation. Substituting for these values, $18.64-20.76=-2.12$, which is in close agreement with expectation. Such is to be expected since almost all of the χ^2 values for individual months were low, only three causing any suspicion of significant departures from expectation, October, 1912, July, 1919, and January, 1921, in which the χ^2 values were respectively 6, 4, and 8. It is, of course, possible that variations in the sex ratio might be associated with the season and still such might not show wider departures from normal expectation than would be expected by chance.

BIRTH WEIGHTS AND GAINS

The birth weights are expressed at birth and gains are expressed 33 days after birth, but both are shown in Figure 9 for the month in which the young were born. The variations shown in the figure were similar in most years. This is probably due to the fact that the gains made in a certain month are evidently affected by the same influences that act on the fetus in utero and affect the birth weights of the individuals born in that month, although there is some indication that birth weights are a little slower in reacting than gains.

Both birth weights and gains show a rather regular cycle over most of the years, the low points being in the winter and the high points in the late spring or early summer (May and June). The years of unfavorable conditions, 1915, 1916, 1917, and 1918, are particularly prominent in these data. With some zigzagging, birth weights and gains show a rapid decline beginning approximately with December, 1914. In the favorable year, 1910, the customary drop in the late winter and early spring was much abbreviated.

MORTALITY

As in the case of the weights and gains, the percentage of animals born alive and the percentage raised of those that were born alive are recorded for the month in which the individuals were born, and are closely related, generally showing low points in the winter or early spring and high points in the summer or fall. In April the curve for percentage born alive was frequently low, while in July it was high. As in the gains, the percentage raised is affected by the same conditions that affect the percentage born alive; i. e., while the young are in utero the mortality is increased in the same way that the mortality of individuals between birth and weaning is affected. The fluctuations in the percentage raised are somewhat greater than the fluctuations in the percentage born alive, probably because for young in utero the dam acts somewhat as a buffer to immediate changes in the environment.

NET FERTILITY

The net fertility is a combination of four of the other characters and therefore should show somewhat the same general course. It is essentially the product of the litters per 100 matings, size of litter, percentage born alive, and percentage raised of those born alive, except that the two latter indices were corrected to eliminate the effects

of size of litter and sex. The general course of the net fertility curve is low late in the winter and early spring, reaching a peak in midsummer or early fall.

AVERAGES FOR COMBINED MONTHS

In the study of the seasonal relations of the indices, the data for all months of January, all months of February, etc., were combined and treated as one year. This was virtually the weighted averages for each of the months. In this way the indices for each of the combined months are based on large numbers of individuals, the smallest number in any month being 630 litters and 1,973 individuals in February. The records for the combined months are given in Tables 8 and 9, together with comparisons for the averages calculated, corrected and uncorrected weights, and mortality percentages being used. This was done for the purpose of estimating the effect of the correction factors used on the several groups of data. As is quite evident, eliminating the effect of size of litter and sex on weights and mortality has had little effect on the relative ranking of different months for a particular index, but it has tended to bring out more strikingly the variations.

TABLE 8.—*Averages for fertility characters in combined months, 1906 to 1924 (litters of 1–6)*

Month	Matings	Litters	Guinea pigs	Litters per 100 matings	Size of litter	Sex ratio	Born alive		Raised to weaning of those born alive	
							Corrected	Uncorrected	Corrected	Uncorrected
	Number	*Number*	*Number*	*Number*	*Number*	*Per cent Males*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
January	3,738	979	2,400	26.19	2.45	52.45	78.28	79.45	73.60	74.03
February	3,692	830	1,973	22.48	2.38	52.19	76.87	78.01	69.22	69.62
March	3,695	1,055	2,563	28.55	2.43	51.79	77.60	78.60	71.02	71.40
April	3,591	844	2,085	23.50	2.47	47.76	80.38	81.11	82.00	82.35
May	3,491	904	2,224	25.90	2.46	50.52	85.21	85.57	87.67	87.85
June	3,541	1,061	2,734	29.96	2.58	50.48	88.12	87.99	90.06	90.03
July	3,540	1,053	2,924	29.75	2.78	49.41	90.08	88.33	88.88	88.20
August	3,632	1,076	3,032	29.63	2.82	49.60	87.00	85.40	85.87	85.27
September	3,651	919	2,405	25.17	2.62	52.47	83.65	83.52	85.53	85.48
October	3,773	1,094	2,916	29.00	2.67	51.32	81.26	80.83	83.07	82.88
November	3,917	1,028	2,715	26.24	2.64	49.03	84.26	84.36	83.83	83.89
December	3,899	1,080	2,729	27.70	2.53	50.39	79.66	80.27	78.80	79.09
Average	[a]44,160	[a]11,923	[a]30,700	27.00	2.57	50.58	83.01	83.01	82.30	82.30

[a] Total.

Size of litter tends to affect the influence of other factors on weights and mortality; for instance, if conditions are unfavorable litters will be small in size, but as a result of this birth weights will be correspondingly larger, etc. The elimination of the effects of sex has been relatively unimportant because the sexes were nearly equally distributed in the combined months, but this no doubt had an important effect in individual months where the numbers were smaller and the sex ratios showed more variation. The data for the combined months, as presented in Figure 10, afford a better opportunity for studying the average variations of the different indices with the seasons than does Figure 9, which shows the variations over the entire period.

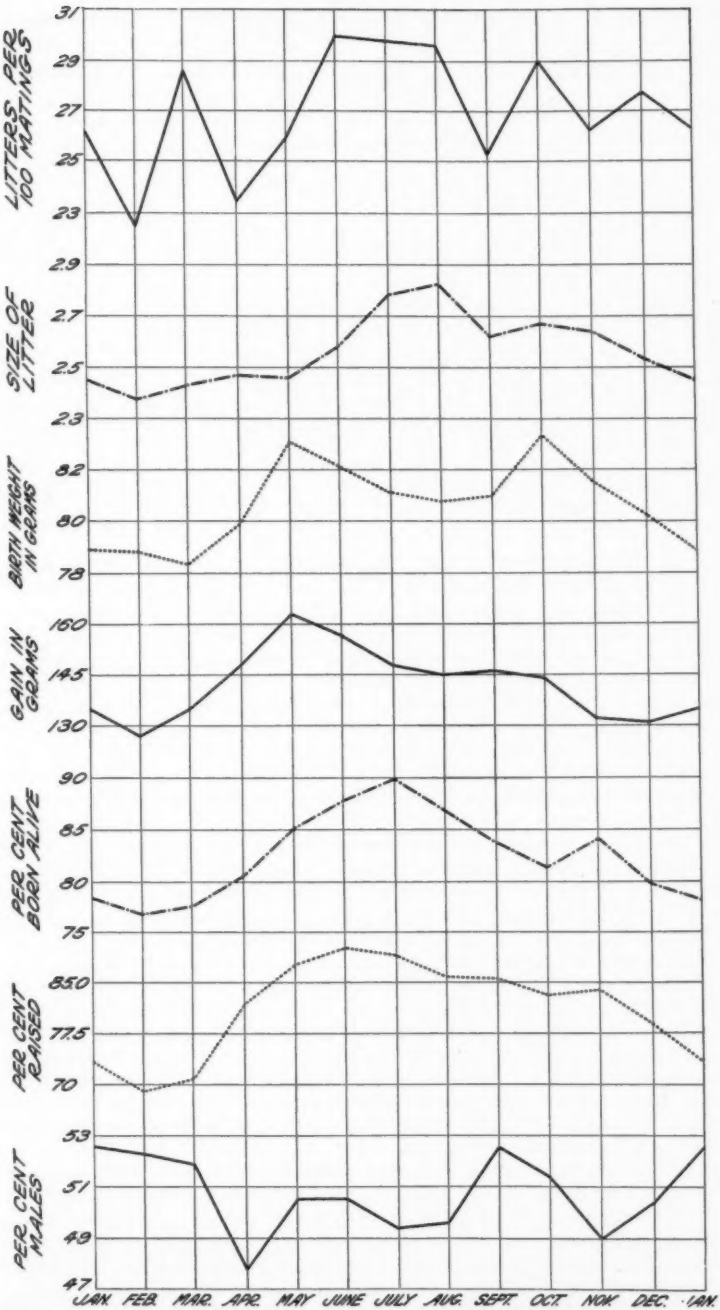

FIGURE 10.—Monthly indices for combined months (litters of 1–6). The month of December gives the mean for each index for all individuals born in December from 1906 to 1924, etc.

TABLE 9.—*Averages for birth and weaning weights and gains in combined months, 1906–1924 (litters of 1–6)*

Month	Birth weights of animals—						Weaning weights		Gains in weight to weaning	
	Born dead		That died before weaning		Raised to weaning					
	Corrected	Uncorrected	Corrected	Uncorrected	Corrected	Uncorrected	Corrected	Uncorrected	Corrected	Uncorrected
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
January	59.57	59.82	70.02	72.19	78.91	80.67	214.17	217.22	135.26	136.55
February	61.13	64.17	69.58	71.81	78.76	80.99	206.67	210.74	127.91	129.75
March	59.62	61.20	68.28	70.14	78.30	80.23	213.54	217.03	135.24	136.80
April	58.53	60.52	67.36	67.85	79.94	80.76	227.64	228.57	147.70	147.81
May	67.07	69.59	72.38	72.55	83.08	83.85	245.80	247.07	162.72	163.22
June	66.07	66.14	70.52	69.35	82.24	82.13	238.75	238.29	156.51	156.16
July	67.03	62.86	66.99	62.51	81.22	79.61	229.49	226.09	148.27	146.48
August	65.56	64.20	69.14	65.81	80.77	78.46	226.03	221.19	145.26	142.73
September	67.38	65.98	72.73	72.16	81.00	80.74	226.82	226.19	145.82	145.45
October	63.55	62.64	72.97	72.12	83.39	82.46	227.51	225.73	144.12	143.27
November	65.37	64.48	72.08	71.26	81.64	81.43	213.58	213.06	131.94	131.63
December	61.86	61.82	72.08	72.68	80.28	81.42	211.01	213.18	130.73	131.76
Average	63.25	63.32	70.26	70.24	81.01	81.03	224.62	224.42	143.61	143.39

It is indeed interesting that litters per 100 matings in the combined months show somewhat the same tendency to alternate in successive months as was observed to a greater extent in the individual months. For the data of the combined months the only case in which the alternating relationship is not shown in successive months is during the summer in June, July, and August, the most favorable season. The beginning of this effect is in May. All other months alternate.

Size of litter for the 6-month period, December to May, inclusive, is below the average, while the six months, June to November, are above the average. The high points for size of litter are in July and August, which appear to be considerably more favorable than other months.

The birth-weight curve is distinctly bimodal, showing high points in the spring and in the fall. Taken together, May and June form the highest mode. The second mode which is the highest for any one month is in October. The tendency for birth weights to rise in the fall was also evident in the curves for the individual months.

The gains showed much the same variation over the year as birth weights, except that the peak in the spring was much more pronounced, and there was but slight evidence of the fall mode. The maximum gains were made by those born during the month of May.

The mortality curves were similar in shape. Both showed low points in December, January, February, and March, followed by a rapid rise to a mode in July for those born alive and to a mode in June for those raised. Particularly large percentages of those born in May, June, and July were raised.

The percentage of males, though the differences are small, shows a high point in January followed by a gradual drop to July and August, except for the low point in April. The percentage of males rises to another high point in September, dropping again to a low point in November. There is some indication of a reciprocal relation of this curve to some of the other indices.

DISCUSSION

Seasonal variation in the breeding of wild animals is common. Under domestication it appears that most classes of livestock breed throughout the year according to the desires of the stockman as to when he wants the young to come, based on likely market demand and when the young can be cared for best. Little consideration is given to the relative number of young, the mortality, or the birth weights and rate of growth of young born in the different seasons of the year.

From the data presented, the summer months, June, July, and August, appear to be on an average the most favorable for frequency of litter, size of litter, and percentage of guinea pigs born alive. The percentage raised of those born alive in these months is also high, but for this group the high period begins for those born in May. Definite peaks in the birth weight and gain curves point to May as the optimum month for birth. Most of the indices are generally high during the summer and early fall; birth weights show a second mode for guinea pigs born in October.

The months of December, January, February, and March were, on an average, generally the most unfavorable for the expression of the indices, a single high point in the frequency of litters in March being the exception.

Since the expressions of the various indices are highest during the summer the favorable environmental conditions that caused such expression, particularly in frequency and size of litter, must have occurred from two to three months earlier; that is, in the spring, probably in the early spring. This assumes that the reaction in frequency of litter is almost immediate, while the number of ova produced, which is the main factor in size of litter, requires a few days longer to be affected. The expression of birth weights is somewhat earlier but gives ample time for the dam to be influenced and still transfer the effect to the young in utero during the last half of the gestation period, this being the time when the major part of the growth of the fetus occurs and when the variations in the birth weights would most likely result. Gains appear to be more closely associated with the birth weights of the individual than with temporary environment as shown by the parallelism of these two curves. Percentage raised either comes closest to being an expression of conditions after birth as it is among the first to show a sharp rise, or it is affected by conditions quite different from those that influence the other indices.

In considering the monthly variations one should not overlook the fact that the basic data for the calculations are taken from the records of several different families, which no doubt react differently to a specific influence. Further, the data for the combined months can not be thought of as representative of any particular year but may be taken as an average expression of conditions in the seasons for the 18-year period. Variations in temperature, sunshine, rainfall, or other meteorological conditions within and without the heated colony house cause extreme variations from the normal, which may not be repeated in successive years, but it is felt that fairly accurate factors for eliminating the effect of season may be obtained from the combined data for general purposes or for those records for particular months of particular years as may be desired.

The discussion of the seasonal variations in the indices would not be complete without reference to the sex ratio. The variations in the individual months are so irregular that they are related to the other indices with some difficulty, but in the combined months the percentage of males is high in January, February, and March, when other indices are low, and high in September and October, when the indices for mortality and gains are dropping. This would point toward a higher prenatal mortality of females when conditions are poor, which is not in conformity with the general belief of greater prenatal mortality of males. With the large amount of data on which this study is based and the fact that many of the differences, particularly the larger ones, are more than three times the probable error, it seems proper to conclude that there were significant seasonal variations in the sex ratio in the guinea-pig data studied.

CORRELATIONS OF MONTHLY INDICES

The primary object in determining the monthly indices was for calculating the relation between the various measurable components of fertility and related factors over several months. Simple correlations were calculated between the eight monthly indices within the same month and for each one with each of the others for the four preceding and four succeeding months, using as a basis the monthly indices from December, 1906, to December, 1924. The correlations thus indicated the relationship between the different indices over a 9-month period.

The correlation coefficients calculated in this way are presented in Table 10 and in Figures 11 to 16. The month designated as 0 refers to the basic month for the group. For example, the correlations of litters per 100 matings with the other indices are given for litters per 100 matings born in the 0 month as related to itself, size of litter, etc., in each of the other months. Those at the left of the 0 month, designated by minus signs, refer to months preceding, and those at the right, designated by plus signs, refer to months succeeding the 0 month.

The index to which each curve in the figures applies is indicated on the margin. The distance between the horizontal lines represents values from 0 to 1.00 on the correlation scale.

As was previously explained, the indices for gains and percentage raised of guinea pigs born alive were recorded for the month in which the guinea pigs were born but were really expressed 33 days later. For this reason in the plotting of the correlation coefficients these indices have been advanced 33 days in their relation to the other indices, and vice versa. In Table 10 they have been advanced one month to show more clearly the relation between the expression of each index. Net fertility was calculated for the guinea pigs born in a certain month and therefore applies to a combination of conditions before birth and 33 days after birth. It, therefore, does not express conditions at birth nor conditions during the succeeding month. Its relation to the other indices is presented, however, as it is the net measure of fertility, being the number of young raised from those born in a particular month per 100 matings in that month. Any other means of calculating an index of this sort appeared to be subject to similar or greater criticism.

TABLE 10.—*Correlation coefficients between the various indices*

[Values in the 0 month as correlated with the various monthly indices over a 9-month period]

LITTERS PER 100 MATINGS

Index	Correlation coefficient when the preceding or succeeding month is—										
	−5	−4	−3	−2	−1	0	+1	+2	+3	+4	+5
Litters per 100 matings		+0.029	+0.133	+0.120	−0.042	+1.000	−0.042	+0.120	+0.133	+0.029	
Size of litter		+.060	+.152	+.087	+.207	+.460	+.301	+.259	+.114	+.142	
Birth weights of those raised		−.003	+.009	+.165	+.220	+.204	+.059	+.070	+.137	+.015	
Gain to weaning			+.008	+.205	+.369	+.274	+.206	+.123	+.050	+.061	−0.070
Percentage born alive		+.070	+.135	+.156	+.397	+.298	+.198	+.051	+.065	−.014	
Percentage raised of those born alive			+.101	+.128	+.345	+.384	+.195	+.133	+.074	−.002	−.045
Net fertility		+.117	+.179	+.259	+.268	+.718	+.173	+.192	+.166	+.034	
Percentage males		+.107	−.196	−.017	−.130	−.048	−.128	−.032	−.008	+.054	

SIZE OF LITTER

Litters per 100 matings		+0.142	+0.114	+0.259	+0.301	+0.460	+0.207	+0.087	+0.152	+0.060	
Size of litter		+.301	+.293	+.429	+.563	+1.000	+.563	+.429	+.293	+.301	
Birth weights of those raised		+.290	+.429	+.547	+.552	+.417	+.352	+.347	+.296	+.189	
Gain to weaning			+.443	+.628	+.690	+.554	+.397	+.278	+.197	+.075	+0.036
Percentage born alive		+.241	+.345	+.543	+.512	+.470	+.241	+.129	+.024	+.068	
Percentage raised of those born alive			+.345	+.488	+.591	+.594	+.428	+.315	+.185	+.095	+.014
Net fertility		+.355	+.415	+.611	+.658	+.743	+.440	+.271	+.217	+.155	
Percentage males		−.073	−.124	−.159	−.082	−.113	−.020	−.019	+.009	−.069	

BIRTH WEIGHTS OF THOSE RAISED

Litters per 100 matings		+0.015	+0.137	+0.070	+0.059	+0.204	+0.220	+0.165	+0.009	−0.003	
Size of litter		+.189	+.296	+.347	+.352	+.417	+.552	+.547	+.429	+.250	
Birth weights of those raised		+.468	+.534	+.606	+.753	+1.000	+.753	+.606	+.534	+.468	
Gain to weaning			.460	+.504	+.605	+.747	+.701	+.510	+.381	+.305	+0.217
Percentage born alive		+.218	+.302	+.285	+.323	+.435	+.417	+.213	+.101	+.057	
Percentage raised of born alive			+.319	+.319	+.409	+.500	+.538	+.462	+.306	+.176	+.153
Net fertility		+.246	+.326	+.314	+.388	+.518	+.509	+.401	+.217	+.146	
Percentage males		+.008	−.063	−.083	−.094	−.119	−.118	−.016	−.009	+.023	

GAINS TO WEANING

Litters per 100 matings	−0.070	+0.061	+0.050	+0.123	+0.205	+0.274	+0.369	+0.205	+0.008		
Size of litter	+.036	+.075	+.197	+.278	+.397	+.554	+.690	+.628	+.443		
Birth weights of those raised	+.217	+.305	+.381	+.510	+.701	+.747	+.605	+.504	+.460		
Gain to weaning		+.254	+.364	+.524	+.757	+1.000	+.757	+.524	+.364	+0.254	
Percentage born alive	+.113	+.223	+.344	+.412	+.551	+.618	+.504	+.279	+.131		
Percentage raised of those born alive		+.153	+.223	+.361	+.537	+.694	+.695	+.515	+.332	+.209	
Net fertility	+.061	+.189	+.276	+.439	+.601	+.684	+.669	+.470	+.235		
Percentage males	+.101	−.003	−.012	−.103	−.162	−.121	−.108	−.064	+.017		

PERCENTAGE BORN ALIVE

Litters per 100 matings		−0.014	+0.065	+0.051	+0.198	+0.298	+0.397	+0.156	+0.135	+0.070	
Size of litter		+.068	+.024	+.129	+.241	+.470	+.512	+.543	+.345	+.241	
Birth weights of those raised		+.057	+.101	+.213	+.417	+.435	+.323	+.285	+.302	+.218	
Gain to weaning			+.131	+.279	+.504	+.618	+.551	+.412	+.344	+.223	+0.113
Percentage born alive		+.055	+.086	+.300	+.476	+1.000	+.476	+.300	+.086	+.055	
Percentage raised of those born alive			+.062	+.192	+.447	+.595	+.643	+.454	+.306	+.195	+.124
Net fertility		+.050	+.138	+.301	+.509	+.702	+.583	+.418	+.289	+.184	
Percentage males		+.056	+.054	−.018	−.118	−.230	−.044	−.012	+.023	+.061	

TABLE 10.—*Correlation coefficients between the various indices*—Continued

PERCENTAGE RAISED OF THOSE BORN ALIVE

Index	Correlation coefficient when the preceding or succeeding month is—										
	−5	−4	−3	−2	−1	0	+1	+2	+3	+4	+5
Litters per 100 matings	−0.045	−0.002	+0.074	+0.133	+0.195	+0.384	+0.345	+0.128	+0.101		
Size of litter	+.014	+.095	+.185	+.315	+.428	+.594	+.591	+.488	+.345		
Birth weights of those raised	+.153	+.176	+.306	+.462	+.538	+.500	+.409	+.319	+.319		
Gain to weaning		+.209	+.332	+.515	+.695	+.694	+.537	+.361	+.223	+0.153	
Percentage born alive	+.124	+.195	+.306	+.454	+.643	+.595	+.447	+.192	+.062		
Percentage raised of those born alive		+.117	+.223	+.417	+.727	+1.000	+.727	+.417	+.223	+.117	
Net fertility	+.061	+.159	+.312	+.511	+.706	+.724	+.562	+.328	+.211		
Percentage males	+.126	+.074	−.045	−.134	−.118	−.102	−.075	−.028	+.044		
	NET FERTILITY										
Litters per 100 matings		+0.034	+0.166	+0.192	+0.173	+0.718	+0.268	+0.259	+0.179	+0.117	
Size of litter		+.155	+.217	+.271	+.440	+.743	+.658	+.611	+.415	+.355	
Birth weights of those raised		+.146	+.217	+.401	+.509	+.518	+.388	+.314	+.326	+.246	
Gain to weaning			+.235	+.470	+.669	+.684	+.601	+.439	+.276	+.189	+0.061
Percentage born alive		+.184	+.289	+.418	+.583	+.702	+.509	+.301	+.138	+.050	
Percentage raised of those born alive			+.211	+.328	+.562	+.724	+.706	+.511	+.312	+.159	+.061
Net fertility		+.203	+.337	+.486	+.616	+1.000	+.616	+.486	+.337	+.203	
Percentage males		+.074	−.098	−.062	−.155	−.167	−.102	−.040	+.008	+.015	
	PERCENTAGE MALES										
Litters per 100 matings		+0.054	−0.008	−0.032	−0.128	−0.048	−0.130	−0.017	−0.196	+0.107	
Size of litter		−.059	+.009	−.019	−.020	−.113	−.082	−.159	−.124	−.073	
Birth weights of those raised		+.023	−.009	−.016	−.118	−.119	−.094	−.083	−.063	+.008	
Gain to weaning			+.017	−.064	−.108	−.121	−.162	−.103	−.012	−.003	+0.101
Percentage born alive		+.061	+.023	−.012	−.044	−.230	−.118	−.018	+.054	+.056	
Percentage raised of those born alive			+.044	−.022	−.075	−.102	−.118	−.134	−.045	+.074	+.126
Net fertility		+.015	+.008	−.040	−.102	−.167	−.155	−.062	−.098	+.074	
Percentage males		+.076	−.102	+.008	+.047	+1.000	+.047	+.008	−.102	+.076	

In the presentation of correlation coefficients the probable errors are not given, but they are easily estimated. The correlations are based on 213 months' records in all cases, the first four months' indices which were calculated from smaller numbers of individuals only being used in the correlations with indices in months preceding and succeeding the 0 months. The formula for the probable error for the simple correlation coefficient is $0.6745\frac{1-r^2}{\sqrt{n-2}}$, where r is the correlation coefficient and n is the number of pairs correlated. The probable error of any coefficient may thus be determined by multiplying $1-r^2$ by 0.046. The probable error is 0.046 for correlations from 0.00 to 0.10 and is reduced for larger coefficients.

It will be noted that in general the nine correlations between the indices of a particular character and those for indices of another character in nine successive months form curves starting with lower values and gradually rising to higher values, dropping off toward the end to lower values. For the purpose of final analysis it was desired to know the point at which the maximum relation between different

indices occurs, which makes necessary an estimation of the mode of each curve of the correlation coefficients. A thoroughly satisfactory method of estimating the true course of the curves was not evident.

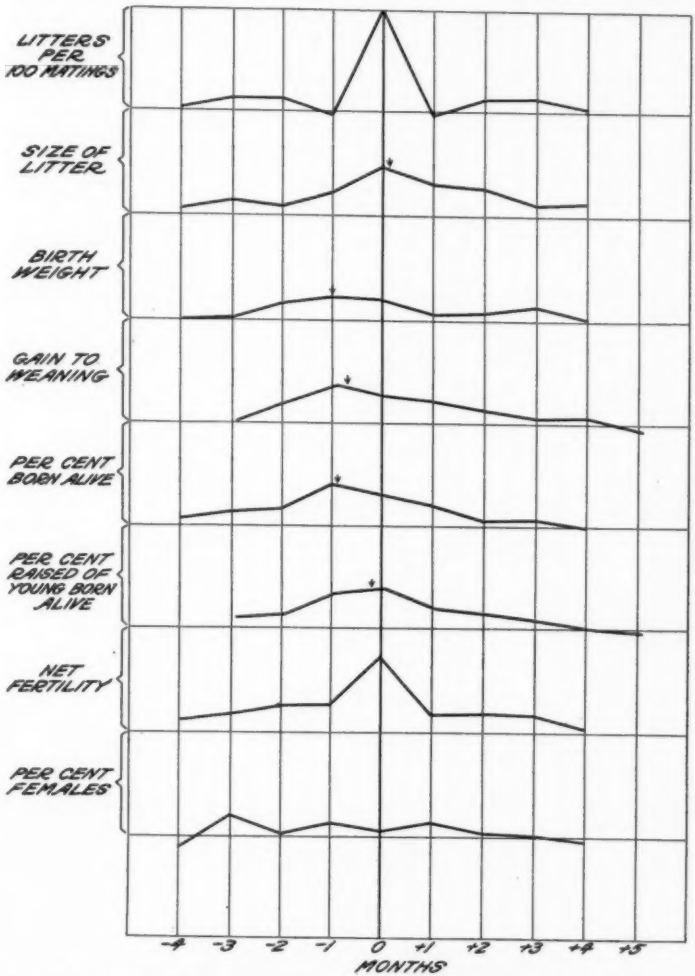

FIGURE 11.—Correlations between litters per 100 matings and the other indices in successive months, designated −4 to +5. The 0 months refer to happenings in the same month, while the correlations in −1, −2, −3, etc., 7 months are for the correlations between happenings in the first, second, and third preceding months, and frequency of litter, in the 0 month. Likewise, the correlations between happenings in the first, second, third, etc., succeeding months and the frequency of litter in the 0 month are indicated by +1, +2, +3, etc.

One method considered was to fit some mathematical formula to each, but the question arose as to which points at the ends of each curve should be omitted and what equation should be used that would obviously be different for each curve. The choice of such a method

is purely arbitrary, and the measure of success is the judgment of the eye. It therefore seemed as well to attempt to fit by the eye in the first place, when so much irregularity existed.

The correlations are briefly discussed below, first with reference to the more significant ones and finally with reference to the relationship

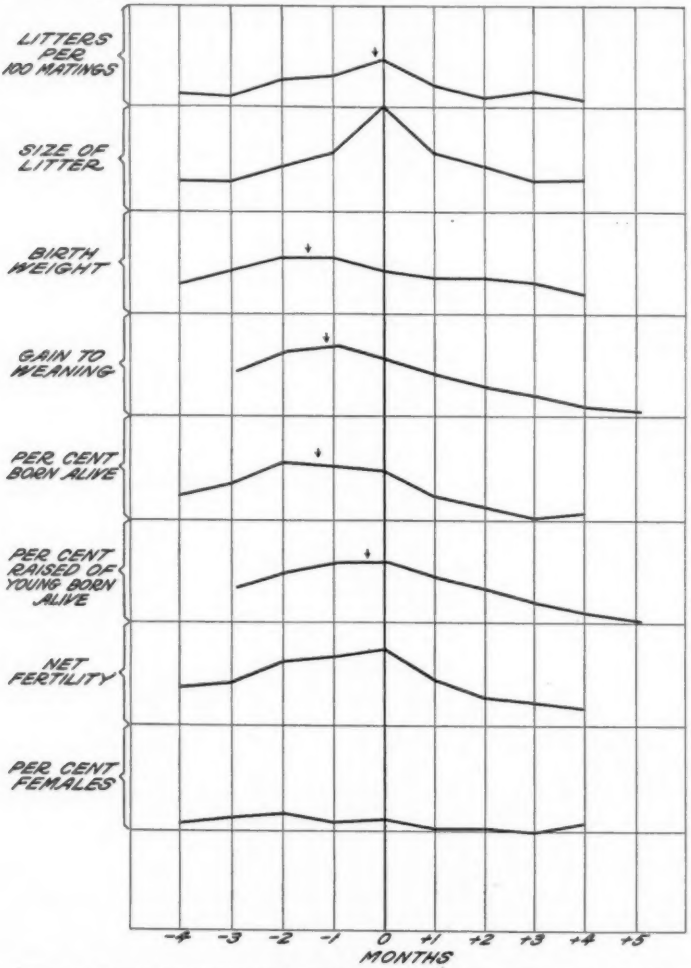

FIGURE 12.—Correlations between size of litter and other indices in successive months. Explanation as of Figure 11

and time interval between expressions of maximum relationship from which estimates of the major influences of common conditions on the different indices were made. The interpretation of the correlations must be made on the basis of related reaction to simultaneous or related conditions and not on a cause-and-effect basis, since there is no apparent reason for such a relationship in most cases.

LITTERS PER 100 MATINGS

The correlations between litters per 100 matings and the other indices were not entirely satisfactory because of the irregular behavior of this index from month to month. This irregularity resulted in generally

FIGURE 13.—Correlations between birth weights and other indices in successive months. Explanation as of Figure 11

low correlations, the highest being 0.46 in the 0 months, with size of litter, followed closely by 0.40 for percentage of young born alive in the −1 month and 0.38 for percentage raised in the 0 month.

It is quite obvious that both frequency and size of litter are largely determined at the time of conception, but the influences must also be

operative before conception, and both may be modified during gestation, frequency of litter by abortions and complete resorption of litters, and size of litter by resorption of individuals. The same environmental effects may, however, not operate at the same time on both

FIGURE 14.—Correlations between gains and other indices in successive months. Explanation as of Figure 11

indices and the two may respond differently. There is some indication of difference in response, as the curve of the correlation coefficients between the two is maintained higher for the following (+) months than for preceding (−) months. This departure from symmetry appears to be about one month. This thus indicates that size

of litter, while evidencing the maximum effect of a particular influence in the same month as frequency of litter, is somewhat slower in its reaction, showing more lag in its general response. The lag of the maximum correlation between frequency and size of litter was estimated from the mode of the correlation curve at +5 days. Assuming

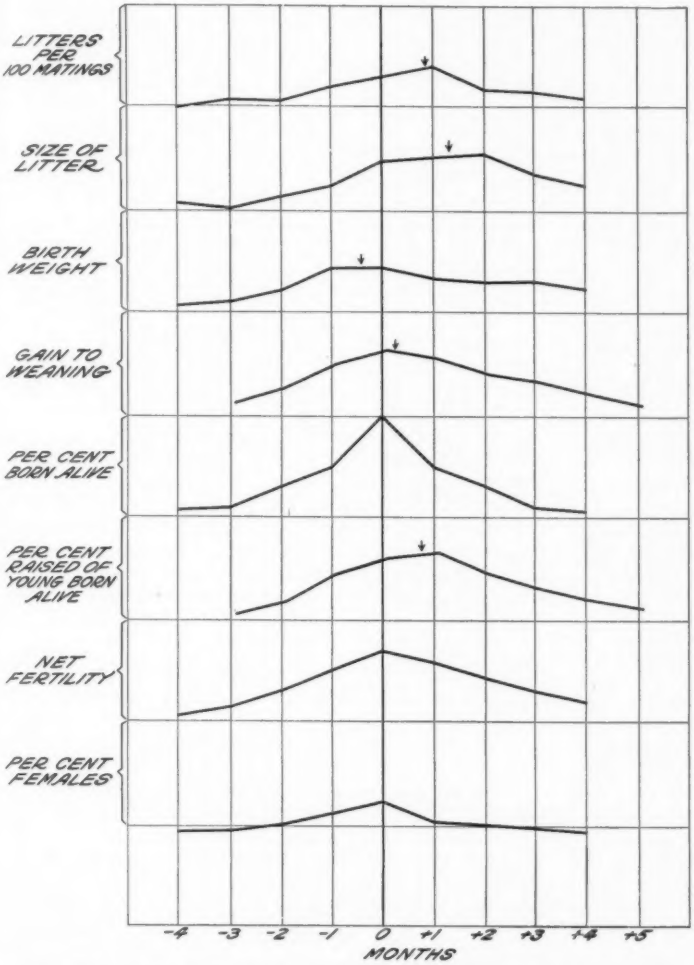

FIGURE 15.—Correlations between percentage born alive and other indices, in successive months. Explanation as of Figure 11

that the latest that frequency of litter could be influenced would be at conception, size of litter, being five days slower in responding, would be affected in litters conceived five days later. Thus conditions associated with frequency at conception are associated with the number of ova produced by females conceiving five days later.

As one would expect, the percentage of young born alive reacts somewhat more promptly to a common influence than frequency of litter. It is readily understood how an extremely unfavorable environmental condition, such as extreme cold, acting at birth, might

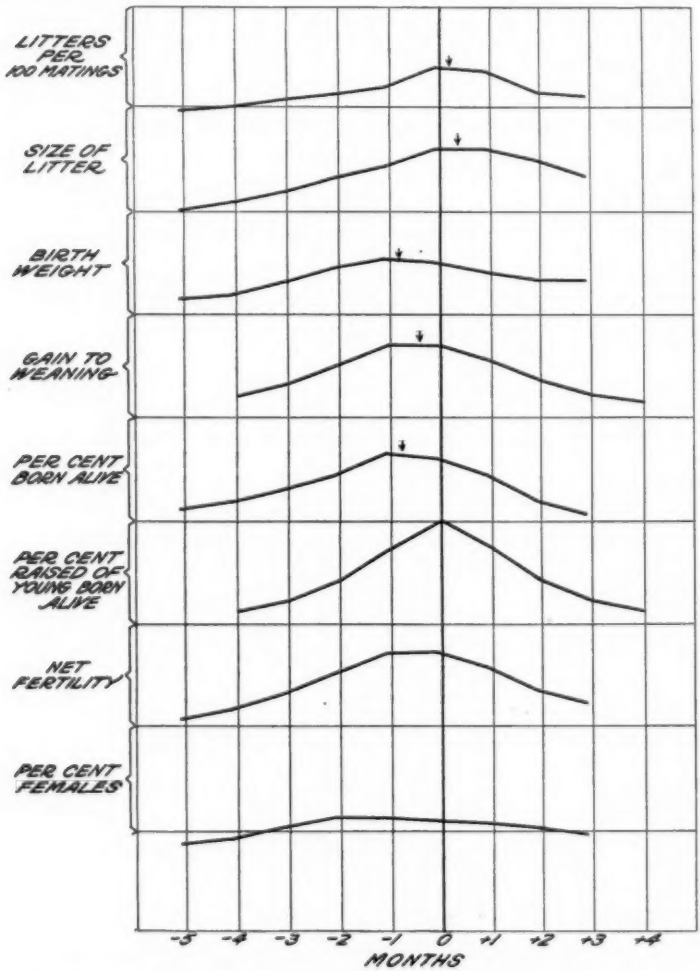

FIGURE 16.—Correlations between percentage raised of guinea pigs born alive and other indices in successive months. Explanation as of Figure 11

destroy entire litters. However, as indicated by the correla ion coefficients, the reaction to such immediate conditions is not of primary importance, or the time relations would be more widely separated than one month. Three of the five significant correlations of frequency of litter and percentage born alive are with percentage

born alive before the 0 month, with the estimated mode of the maximum relation 26 days before the time of expression of frequency of litter. In calculating back it is found that the young expressing the maximum relation of percentage born alive to litters per 100 matings were at the twenty-sixth day of the gestation period[5] when the litters by which frequency was measured were conceived. Thus, it may be assumed that the maximum concomitant influences on frequency of litter and percentage born alive occurred, respectively, at conception and at the twenty-sixth day of gestation. The course of the curve showing more lag in the 0 and + months than in the − months indicates that the influence on percentage born alive is greater prior to the twenty-sixth day of gestation than after that point is reached.

The mode of the correlation curve between frequency of litter and percentage of young raised of those born alive was estimated at −5 days. The percentage raised is expressed 33 days after birth; therefore, the guinea pigs expressing percentage raised are born 38 days before those expressing frequency of litter. Therefore, at the time those expressing frequency of litter are conceived (−68 days) those expressing percentage raised are at the thirty-eighth day of the gestation period.

The correlations of frequency of litter with birth weights and gains were relatively low. It is, however, apparent that the mode for the relation of frequency of litter to the weights and gains occur somewhere during the −1 month, and the points were estimated for gains at −20 days and for birth weights at −29 days. As gains were expressed at 33 days of age, the young which showed the effect of gain 20 days before the effect on frequency was apparent were 33 days old. The influence must have operated 68 days before the litters expressing frequency were born. It therefore acted on gain 68 − (20 + 33) = 15 days before birth, or at the fifty-third day of gestation. Similarly, from the concomitant variations between frequency of litter and birth weights the influence acted on birth weights at the twenty-ninth day of gestation.

SIZE OF LITTER

Size of litter, unlike frequency of litter, shows a relatively high correlation with itself in successive months, and with the other indices except frequency of litter. (Table 10 and fig. 12.) It not only maintains a high correlation between the first and second succeeding months, +0.56 and +0.43, but for three and four months away the correlation is practically +0.30. This seems to indicate that size of litter is the result of an accumulation of environmental effects or that the environmental factors affecting size of litter are associated.

Because of the delayed evidence of the size of litter—at the end of the 68-day gestation period—most of the higher correlations were in either the 0 or − months. The highest correlations were +0.69 and +0.63, with gains in the −1 and −2 months; +0.59 with percentage raised in each of the 0 and −1 months; +0.54 and +0.51 with percentage born alive in the −2 and −1 months; and +0.55 with birth weights in each of the −1 and −2 months, respectively.

The 9-month period for which the correlations were calculated was not sufficient to bring out all the existing relationships between size of litter and the other indices, particularly those for birth weight,

[5] The gestation period varies from 65 to 71 days, but for convenience it has been here considered as 68 days.

gains, and percentage of young raised of those born alive. Likewise, there were correlations of +0.30 between the average size of the litters born six months apart.

The correlation curves for size of litter were smoothed and the modes estimated, as shown in Figure 12, in the same manner as for the frequency-of-litter correlations. From the modes of these curves, it was estimated that variations in the other indices, showing the maximum degree of association with variations in size of litter, were expressed the following number of days prior to the birth of the litter from which size of litter was measured: Frequency of litter, 5 days; birth weight, 45; gain, 34; percentage of young born alive, 39; and percentage raised, 10 days. If it requires five days before conception for an influence to act on size of litter (see p. 151), the common influence on size of litter and the other indices may be calculated to have their maximum effect on frequency of litter at conception, and on the other indices at the following stages of gestation: Percentage born alive, 34 days; birth weights, 40; percentage of young raised, 38; and gains, 62 days.

WEIGHTS

Birth weights correlated with birth weights and gains correlated with gains over the 9-month period present similar curves, except that the relationship between birth weights evidently extends over a longer time. The correlation for birth weights separated over a period of four months is +0.47, while the correlation for gains over a similar period is +0.25. The correlation between birth weights in the succeeding months was +0.75 and between gains in succeeding months +0.76. The correlations between birth weight and gain are relatively very high. The correlation between the birth weights and the gains made by the same individuals, +0.70, was not quite so large as the correlation between the birth weights and gains made at the same time, +0.75, by different individuals.

There were relatively high correlations of both birth weights and gains with the other indices in several months, as shown in Table 10 and Figures 13 and 14. There was no index, except litters per 100 matings, which did not in some months show a correlation above +0.40 with birth weight and gain.

Several of the birth-weight-correlation curves showed a bimodal tendency, or at least were very flat. This was particularly pronounced for the correlations with percentage of young born alive, frequency of litter, and size of litter, and there appeared to be some indication of such a relationship with percentage raised and gain. This may be explained as due to the bimodal characteristic of the birth-weight index noted when the seasonal variations in the combined months were discussed.

The gain correlation curves were somewhat steeper than those for birth weight, otherwise the relationships were similar, though gains in successive months were not quite so closely correlated.

The modal relation between the manifestation of characteristics showing maximum relations were, with birth weights as a base, at +29 days for frequency of litter, +45 days for size of litter, +14 days for gains, +12 days for percentage of young born alive, and +25 days for percentage raised. The modes of the gain correlation curves were established at +20 days for frequency of litter, +34 days for size of litter, −14 days for birth weight, −8 days for percentage of young

born alive, and +13 days for percentage raised. The modal relation between birth weights and the other indices thus occurs from 9 to 20 days later than the modal relation between gains and the same indices.

The weight curves bring out the similarity of the reaction of intrauterine and extrauterine life to the environment, but the birth-weight and gain relationships with the other indices are distinctive in the relatively greater tendency for the birth weights to be affected over a longer period. A comparison of the correlations between indices expressed at birth with those expressed at weaning shows that there is generally greater correlation between expressions in the same month than between the indices for the same individuals expressed in successive months.

MORTALITY

In contrast with most of the curves for the correlations with birth weights, the 9-month period covers practically the entire time over which there are significant relationships for percentage of young born alive and percentage raised of those born alive, both with themselves and with other indices. (Table 10 and figs. 15 and 16.) The curve that shows percentage born alive correlated with itself is particularly steep, and the significant relations cover five months, i. e., two months in either direction from the 0 month. The significant relations between the percentage raised of those born alive in successive months covers about seven months, but the relation is dwindling out pretty rapidly two months away. The curves for the correlation of percentage born alive and percentage raised with the other characteristics are similar in shape, and the values are relatively uniform, except that those for percentage raised are somewhat higher and the curves are broader. The highest correlation between the mortality percentages was 0.64 for the same litters; i. e., percentage born alive in the 0 month and percentage raised in the +1 month. Evidently, if conditions were favorable for low mortality at birth, a relatively large percentage survived to weaning.

Unlike the weight correlation curves, there were several indications of closer relationship between the monthly indices for the same individuals expressed at different times than for those of different individuals expressed at the same time.

It is curious that percentage of young born alive was not so closely correlated with itself in succeeding months as it was with certain of the other indices in those months. There were significantly higher correlations for four months with percentage raised, three months with gains, and two months with size of litter. Excluding litters per 100 matings, there were correlations above +0.5 in two or more months between the mortality percentages and all the other indices, except for the relationship between birth weight and percentage of young born alive, for which there were two months above +0.4.

The correlations with percentage of young born alive formed sharper peaked curves than the correlations with percentage raised, though the correlations with the latter in the months of maximum relation were frequently a little larger. The difference between the expression of the modes of the percentage born alive and percentage raised curves when related to each other, was estimated at 23 days. If all of the indices were associated with the expression of the same factors, similar differences would be expected between the mode of

the correlation curves for (1) percentage born alive–frequency of litter and percentage raised–frequency of litter curves, and (2) percentage born alive–size of litter and percentage raised–size of litter curves, etc. This is approximated. The modes for the different index correlation curves with percentage born alive and percentage raised were, respectively: Frequency of litter, +26 and +5 days; size of litter, +39 and +10 days; birth weights, −12 and −25 days; and gains, +8 and −13 days. The differences thus range from 13 to 29 days.

NET FERTILITY

Net fertility is very closely related, mathematically, to litters per 100 matings, size of litter, percentage of young born alive, and percentage raised of those born alive; and the correlation coefficients calculated were +0.70 or more in all four cases, in the same month. (Table 10.) It is, however, interesting to note that there are other correlations nearly as great in other months. For example, the coefficient of correlation with percentage raised in the +1 month, +0.71, is not significantly different from the correlation with the data forming a direct component of it. The correlations of net fertility with gains made during the month that the young are raised and during the gestation period, particularly during the last month of gestation, are nearly as large as the correlation between net fertility and other indices forming component parts of it. The correlations with the size of litter for the first two months after the young are born are +0.60 and +0.61. In fact, frequency of litter is the only one of the fertility or weight factors which does not show a relatively high correlation for several months before and after the 0 month.

SEX RATIO

Attention has already been called to the fluctuations in the sex ratio and the indication that these might be related to the seasons and possibly to variations in the other monthly indices. (Figs. 9 and 10.)

In the planning of monthly indices during the course of the study the sex ratio was tabulated as a matter of interest, but with the expectation that no correlation would be found with the other indices. However, among the correlations as a whole nearly all were positive, but of the 63 correlation coefficients between percentage of males and the other indices given in Table 10, there were only 17 which were positive, and all of these were with indices three months or more from the month in which the sex ratio was measured. Twelve were at the extreme end of the period and all were small. The largest positive correlations were +0.10 in the +5 month for gains, +0.13 in the same month for percentage raised, and +0.11 in the +4 month for frequency of litter. There is, at least, one month in which the correlation of the sex ratio with each of the other indices is −0.12 or larger. With litters per 100 matings the alternation of the months in the degree of relationship is quite distinct. The correlations of percentage of males in one month with frequency of litter in the next month was −0.13; and between the sex ratio in one month and frequency of litter in the third following month −0.20. The correlations of percentage of males in one month with size of litter in the same month and in the three succeeding months were −0.11, −0.08,

−0.16, and −0.12, respectively. The correlations with birth weights in the same and in the preceding months were both −0.12 and in the succeeding month −0.09. The relation to gains covers a similar period, except that the correlations were somewhat higher, being −0.11, −0.12, and −0.16, and, in the second month later, −0.10. There are no possible significant correlations with percentage of young born alive except in the same month, which is the highest of the coefficients in this group, −0.23, and in the next following month, −0.12. The three highest correlations with percentage raised are within the same month and during the first and second succeeding months, being −0.10, −0.12, and −0.13, respectively.

Very little would be thought of the small correlations of percentage of males with the other indices if they were not so uniform and all in the same direction, thus indicating that a lower percentage of males is associated with all other indices which are assumed to result from favorable conditions. Percentage of young born alive showed the largest correlation with sex ratio, which is hard to understand since sex was found to be unrelated to mortality at birth. In the different litter sizes the values for the percentage born alive alternated for the sex ratio, and the percentage of the two sexes born alive was practically the same.

Further study of the correlations indicated that the variations in the sex ratio were most closely associated with variations in the percentage of young born alive. As the correlations were all small and there was only about 6 per cent of the variability in the sex ratio associated with the variations in the other indices in the months showing the highest correlations, they do not warrant further discussion.

DISCUSSION

The correlations between the indices ranged in the modal months in most cases from 0.40 to 0.75, indicating from 16 to 56 per cent association in the variations. These are obviously high for biological data so pronouncedly influenced by environmental fluctuations, and particularly when there appears to be an almost complete lack of cause-effect relationship. The curves showed a dwindling of the degree of correlation as the time interval increased, though in some cases the 9-month period employed was clearly not sufficient to get away from a considerable degree of association.

In order to bring out more clearly the interval between the maximum degree of associated variation in the different indices Figure 17 was constructed, showing diagrammatically the interval between the reaction of each pair of indices to a common influence. The intervals in days between the evidence of the maximum relations are indicated. Straight lines show the shortest period between such expressions and angles show the longer intervals. For example, frequency of litter was manifested 5 days before the size of litter, and birth weight 29 days before frequency of litter, but birth weight was evidenced 45 days before size of litter instead of 34 days before it. This was, however, the greatest discrepancy, as the differences in other cases vary from 11 days to complete agreement. Dotted lines were used in the figure where the totals are the same as the sum of the component intervals. For example, frequency of litter was evidenced 5 days before size of litter, and percentage of young raised

5 days before frequency of litter. Since percentage raised was 10 days before size of litter, the last two were connected by a dotted line.

There was in general a close agreement among the different paths for measuring the time of evidencing frequency of litter, percentage raised, gains, and percentage of young born alive, and between frequency of litter, percentage raised, and birth weights, as well as between size of litter, frequency of litter, and percentage raised; and percentage raised, gains, and birth weights. From the fact that limited groups can be selected which show similar relations, and that there are few wide departures from the sum of any paths which may be followed, it is convenient to discuss the associations as though all indices reacted to the same complex of conditions. Such are probably closely associated conditions, but these would not be expected to be identical.

Another matter of interest is the order in which different indices evidence the effects of changed environmental conditions. The data throw light on this question only from the standpoint of associated variations in pairs of indices and do not contribute to the variations in a particular index resulting from a specific influence acting only on that index and having no influence on, or influencing to a minor degree, other indices studied. The duration of the intervals between the expression of the maximum amounts of concomitant variation in the different indices permits the establishment of the order in which the reactions are expressed with considerable certainty. The order of a reaction to a particular stimulus is evidenced successively in (1) birth weights, (2) percentage of young born alive, (3) gains, (4) percentage raised, (5) frequency of litters, and (6) size of litter. This order is maintained for all the indices when considered with reference to any single one, but there is a slight overlapping when considered in relation to several. For example, variations in birth weights associated with frequency of litter are evidenced 29 days before frequency of litter, and size of litter 5 days after frequency of litter; but variations in percentage of young born alive associated with variations in size of litter are evidenced 39 days apart, placing the expression of percentage born alive before birth weight, when the latter is calculated from its relation to frequency of litter, and the former from its relation to size of litter. When birth weight is considered from its direct relation to size of litter it again evidences its variation associated with size of litter before the variations in percentage born alive are apparent.

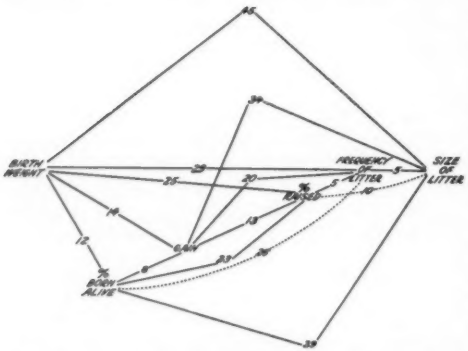

FIGURE 17.—Interval in days between maximum correlations between the expressions of the indices. For explanation see text

Having established the relation between the time of expression of the associated variation in the different indices, it becomes of con-

siderable practical interest to know at what stage in the life history of the animal associated variations in the different indices are most likely to occur. The most promising lead to this is in a consideration of the relation of the various indices to size and frequency of litter. Obviously, these two indices must be mainly influenced before conception, but there appears to be no way of determining from the data just how long before conception. The fact that size of litter requires five days longer to react to certain conditions than frequency of litter helps, but it is not certain that frequency of litter responds immediately, nor is it to be expected that it should. For the want of a better starting point it may be reasoned that frequency of litter is affected at conception and size of litter five days before conception. McKenzie (*9*) found in swine particularly rapid growth of the follicles during prooestrum, with the elimination of some follicles at all times, which lends some support to the selection of such a base, and it is reasonable to believe that the elimination of follicles might be closely associated with environmental conditions.

Assuming that influences must act on size of litter five days before conception, we may estimate the time when the influences act on the other indices. The common influences between size of litter and birth weights would act on birth weights 28 days before birth, or at the fortieth day of the gestation period for birth weight. On the other hand, common influences between frequency of litter and birth weights act on birth weights at the twenty-ninth day of gestation. Similarly, common influences between frequency of litter and percentage of young born alive act on the latter at the twenty-sixth day of gestation, while with size of litter the action is placed at the thirty-fourth day of gestation. Common influences between frequency of litter and gain act on the latter at 15 days before birth, or at the fifty-third day of gestation, and when calculated from size of litter, at six days before birth or at the sixty-second day of gestation. The time of influence on percentage raised is placed at the thirty-eighth day of gestation from its relation to both size of litter and frequency of litters.

The other four indices may now be considered with reference to the time in the gestation period between the action on each. There is 25 days' difference in the expression of percentage of young raised and birth weights. Allowing for the expression of the former at weaning and the latter at birth, this makes percentage raised influenced $33-25=8$ days later in the gestation period than birth weights. Similarly, gains are influenced 13 days later in the gestation period than percentage raised, 25 days later than percentage born alive, and 19 days later than birth weights. These intervals do not give any indication of the time in the life cycle of the animal or the duration of the time between the changes in the environment and the evidence of a reaction of a particular index to it. A threefold relation between two indices and the relation of the basic one to size of litters furnishes an indication of this, but offers some complications. For example, birth weight was estimated to be influenced at the fortieth day of gestation from its relation to size of litter, but at the twenty-ninth day of gestation from its relation to frequency of litter. When birth weight is used as the base calculated from its relation to size of litter, frequency of litter must be influenced 11 days after conception, which seems highly improbable. Similarly, when gain, percentage of young born alive, and percentage raised were employed

as the bases and when the influences acting on them were calculated from size of litter, frequency of litter was influenced 9, 8, and 0 days, respectively, after conception.

With a full appreciation of these small discrepancies, the most satisfactory way to get a clear interpretation of the meaning of the interval between the expression of the reaction of the different indices to common environmental conditions appeared to be by the selection of the most probable single interval between each index based on all the observations. The most probable interval between the expression of the different indices was calculated by the method of least squares, taking account of the 15 equations that could be set up from the intervals given in Figure 17.

The result of this calculation indicated that the different indices expressed their reaction to common influences as follows: First, birth weight, followed in order by percentage of young born alive, 7 days

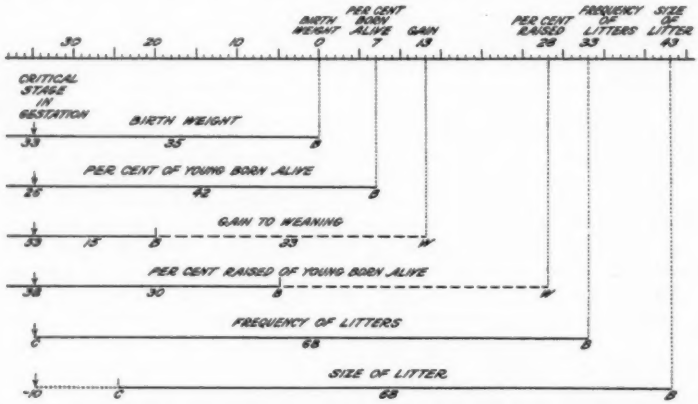

FIGURE 18.—Order of expression of maximum associated variation in the different indices and estimated critical stage in the development of each. The scale at the top represents the interval in days between the expression of the maximum correlations between the indices. In the lower portion of the figure the development of the individuals expressing each index is represented with the estimated critical stage in development for each. The letters C, B, and W refer to conception, birth, and weaning, respectively

later; gain, 13 days later; percentage raised of those born alive, 28 days later; frequency of litter, 33 days later; and size of litter, 43 days after the litters expressing the associated variations in birth weights were born. These relationships are shown diagrammatically on the scale at the top of Figure 18.

It may be assumed that both size and frequency of litter are mainly influenced not later than conception. In the lower portion of Figure 18 the development of the individuals expressing different indices has been diagramed. Birth weights, percentage of young born alive, frequency of litter, and size of litters are expressed at birth, while gain and percentage raised of those born alive are expressed at weaning, 33 days after birth.

From Figure 18 it is apparent that if the maximum influence of the common factors acts on frequency of litter at conception, the critical stage in development for the action on the other indices is 10 days before conception for size of litter and at the following stages in the

gestation period for the other indices: Birth weight thirty-third day, percentage of young born alive, twenty-sixth day, gain to weaning fifty-third day, and percentage raised thirty-eighth day of gestation.

On this basis it may also be estimated that the maximum variations in birth weight associated with the maximum variations in the other indices resulting from a common causal factor, may be expected to occur about 35 days after the operation of the causal factor. If this be correct, the time intervening between the occurrence of the common causal factor and the expression of its effect on the other indices may be estimated as follows: Percentage of young born alive, 42 days; gain to weaning, 48 days; percentage raised of those born alive, 63 days; frequency of litter, 68 days; and size of litter, 78 days.

The time of operation of a particular influence has been referred to as if it were specific, but the breadth of the correlation curves indicates that the influencing conditions extend over a considerable period and are more cumulative than immediate, and some more so than others. Judging from the curves, frequency of litters and percentage of young born alive are more affected by immediate conditions than the others, with size of litter showing the next greatest tendency in this direction. The specific time mentioned should be taken as the latest point in the life cycle at which the maximum influence occurs. It is probable that size of litter and frequency of litter react to influences earlier than those used as a basis. However, Loeb (*8*) showed that there were no large follicles in the guinea-pig ovary after oestrum, and therefore frequency and size of litter would be expected to be the result of conditions acting at some time during the 17-day period prior to the next ovulation. Papanicolaou (*12*) found that ovogenesis in the guinea pig was going on at all times, and that the rate was stimulated by favorable nutrition, seasonal conditions, and oestral activity, and retarded but not stopped by the presence of corpora lutea and competition in the ovary. These findings have a definite bearing on the estimate of the time at which the frequency and size of litters might be influenced, and indicate that the time selected is within the limits of expectation.

There is no doubt that it takes several days, if not weeks or months, in particular cases for conditions to affect a dam's physiological condition so that she will ovulate and conceive, whereas the time relations have been based on this as an immediate effect. Furthermore, size of litter is no doubt affected at least by the beginning of the cycle, culminating in ovulation and the conception of the litter. The constant elimination of follicles from the ovary throughout the various stages in development has been cited by several authors. It thus appears to be a cumulative process, though there may be critical periods with heavy elimination, or times beyond which further atresia is a minor matter. The operation of such influences during the preceding period with an ovulation period intervening would not seem unreasonal.

If seasonal variations in the sex ratio are admitted, of which there seems to be considerable evidence, these variations must be associated with conditions other than those causing the variations in the other indices, with the possible exception of percentage of young born alive; a sex difference in the prenatal mortality at an early stage in gestation was the most plausible means by which the variations in the sex ratio could be brought about.

SUMMARY AND CONCLUSIONS

From a study of the fertility, mortality, birth and weaning weights, and sex ratio in an inbred guinea-pig colony comprising more than 30,000 individuals born during a period of 18 years, the following results were obtained and conclusions reached:

1. Size of litter affected birth weights, weaning weights, and gains to weaning; the weights were larger in all cases for guinea pigs born in the small litters. The reciprocals of the weights were fitted closely by a second-order parabola.

2. Size of litter affected the percentages of guinea pigs born alive and the percentages raised of those born alive; litters of two and three were most favorable but the mortality was considerably greater in the larger litters.

3. Size of litter did not affect the sex ratio of the total young born.

4. The birth weights of those which were raised to weaning age were about 10 gm. heavier than for those which died between birth and weaning. The birth weights of those born dead were approximately 4.5 gm. lighter than those dying between birth and weaning. These differences were maintained constantly throughout the different-sized litters and were statistically significant.

5. Sex was a small but constant factor in influencing birth weights, weaning weights, and gains; males were heavier than females in all litter sizes. The birth weights were similarly affected by sex for those which died between birth and weaning and those raised to weaning, but the difference in those born dead was not significant.

6. The percentage of young born alive was not affected by sex, but over 1 per cent more males than females were raised to weaning of the guinea pigs born alive. This relation was relatively uniform in all litter sizes.

7. Correction factors were developed for eliminating the effect of sex and litter size on the birth weights of guinea pigs born dead, those that died, and those that were raised. Similar correction factors were developed for weaning weights and percentage raised. Correction factors for the effect of size of litter on percentage of young born alive were likewise developed.

8. Monthly indices were calculated, for 217 months, for frequency of litter, size of litter, birth weights, gains to weaning, percentage of young born alive, percentage raised of those born alive, number of young raised per 100 matings, and sex ratio, after making suitable corrections for the effect of size of litter and sex of young.

9. Most of the characteristics showed definite seasonal cycles, conditions being generally unfavorable in the late fall and winter and favorable in the spring and summer. Birth weights showed a bimodal seasonal cycle with higher birth weights in the late spring and fall. There were other exceptions in individual characteristics and for particular years. The sex ratio also showed some seasonal variations.

10. From the simple correlations of each of these characteristics with each other over a 9-month period it was apparent that:

(*a*) Frequency of litter showed the lowest correlation with itself and with each of the other factors, followed in order by size of litter, indicating variations associated with more immediate conditions. This is also true to a less extent of percentage of young born alive. On the other hand, birth weights, gains, and percentage raised of those born alive appeared to result from influences operating over a longer time.

(*b*) It was also apparent that the different indices were probably affected by different influences, but for simplification of explanation the approximate time in the life history of the animal before which the indices are most likely to be affected was calculated from the composite relations as 10 days before conception for size of litter, conception for frequency of litter, twenty-sixth day of gestation for percentage of young born alive, thirty-third day of gestation for birth weight, thirty-eighth day of gestation for percentage raised of those born alive, and fifty-third day of gestation for gain. It was estimated that the reaction to exceptional conditions would be expected to manifest itself first in birth weight, followed in 7 days by percentage of young born alive, in 13 days by gain, in 28 days by percentage raised of those born alive, in 33 days by frequency of litter, and in 43 days by size of litter.

11. From the correlations a small but regular negative association appeared between the percentage of males on the one hand and all the other indices indicating favorable conditions on the other hand.

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THE EFFECT OF ETHYLENE ON THE CHEMICAL COMPOSITION AND THE RESPIRATION OF THE RIPENING JAPANESE PERSIMMON [1]

By W. B. DAVIS and C. G. CHURCH, *Associate Chemists, Laboratory of Fruit and Vegetable Chemistry, Chemical and Technological Research, Bureau of Chemistry and Soils, United States Department of Agriculture*

INTRODUCTION

Although the Japanese persimmon (*Diospyros kaki*) was planted in California as early as 1870 (*18*),[2] no more than 200 acres seem to have been planted as late as 1919. During the 8-year period 1919–1926, however, the bearing acreage more than doubled and the nonbearing acreage increased about sixteenfold, indicating a renewed activity in the industry (*11, 18*).[3]

It is indeed surprising that this industry has not developed more rapidly. The Japanese persimmon is attractive in color, possesses a pleasant flavor, has a high food value, and contains vitamin C (*19*). In addition, disease seldom attacks either the tree or the fruit. The crop enters the market at a time when few other fresh fuits are available. That it keeps well in cold storage is indicated by the fact that near Peking, China, practically the whole winter supply is stored in large outdoor pits (*6*). Some of these desirable qualities have already been pointed out by Gore and Fairchild (*8*), and a more widespread knowledge of them should help to make this fruit more popular.

It is quite probable, however, that the difficulty of removing the astringent taste has been a serious drawback to the wider use of the fruit. In an attempt to eliminate this handicap, the United States Plant Introduction Gardens at Chico, Calif., has introduced a nonastringent variety, the Fuyu. Although its introduction may partly account for increased plantings, the Fuyu has not yet appeared in commercial quantities in the markets of southern California, where the bulk of the industry is located. Since, up to this time, the chief commercial varieties have been astringent, it appears that the removal of the astringency is still of importance.

In spite of the fact that means for removal of the astringency have been known for some time (*8*), and are employed in Japan, they seem as yet to be little used commercially in the United States. With the demonstration that ethylene would decrease the astringent taste and enhance the color (*2*), a new means of astringency removal seemed to be available. That this method is of some practical significance is indicated by the fact that of 550 tons of persimmons estimated as grown in southern California in 1927,[4] 25 tons were treated with ethylene for the local trade.

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[1] Received for publication June 4, 1930; issued February, 1931.
[2] Reference is made by number (italic) to Literature Cited, p. 181.
[3] Calculated from data on trees, estimating 68 trees per acre.
[4] Figures given in a personal communication from the Southern California Persimmon Grower's Association.

In addition to the above-mentioned changes in the color and taste of persimmons brought about by ethylene, preliminary studies (*3*) had suggested that it has a softening effect. Ethylene also seemed to to increase the respiration rate,[5] as it does that of the lemon (*5*), and there appeared to be a relation between the effect of ethylene and the stage of maturity at which treatment is applied, similar to that reported by Rosa (*17*) for the tomato.

Related to the task of removing the astringent taste, therefore, is the determination of the proper stage of maturity at which the fruit should be picked and ethylene applied. Consequently the work reported here was undertaken to determine the effect of ethylene on coloring, softening, chemical composition, and respiration of Japanese persimmons of two varieties at different stages of ripening on the tree and during a short storage period.

PROCEDURE

Samples of the Hachiya, the chief commercial, astringent variety in California, where obtained from an 8-year-old tree on a ranch at Fullerton, Calif. The fruit was picked in such a manner as to get as representative a sample as possible at different stages of maturity. As soon as the samples were picked they were carried by automobile directly to the laboratory. Samples of the nonastringent Fuyu were taken from one tree in the United States Plant Introduction Gardens at Chico, Calif., and sent by express to the laboratory, being 36 to 48 hours in transit. Sampling began September 15, when the fruit was almost wholly green but of nearly full size, and continued at weekly intervals until October 27.

On arrival at the laboratory the persimmons of each variety were divided as uniformly as possible in regard to size and color into four lots of 10 fruits each. The rate of respiration of lots A, B, and C was determined before treatment began, its determination requiring 15 to 22 hours. Lot D was kept at the same temperature as lot B until analyzed, and was therefore the check lot. Data were secured from this lot to determine the effect of temperature and other environmental conditions. After the respiratory rate had been obtained, the untreated lot, A, was analyzed to show maturity changes.

Lots B and C were then exposed to ethylene, 1 part in 1,000 parts, for 46 to 52 hours at room temperature, in large desiccators, which were aired and recharged daily. The respiration rate was then determined for lots B, C, and D. Lots B and D were immediately analyzed, and C was held for observation. Lot B, then, constituted the sample analyzed after treatment. (Table 1.)

[5] DAVIS, W. B. Unpublished data.

TABLE 1.—*Chemical composition of Japanese persimmons, before and after treatment with ethylene*

HACHIYA VARIETY

Sample No. [a]	Date of picking	Astringency [b]	Moisture	Percentage composition on a moisture-free basis					
				Insoluble solids	Soluble solids (by difference)	Total acid [c]	Sugars		
							Reducing [d]	Sucrose	Total
	1927		*Per cent*						
3A	Sept. 20	−−	83.24	23.63	76.37	0.925	46.72	0	46.72
3B	do	−−	83.60	26.95	73.05	1.128	56.59	0	56.59
3D	do	−−	83.17	26.92	73.08	.980	55.56	0	55.56
5A	Sept. 27	−−	82.57	20.37	79.63	.889	62.88	0	62.88
5B	do	−−	82.92	21.95	78.05	.878	63.76	0	63.76
5D	do	−−	82.34	21.86	78.14	.784	64.10	0	64.10
7A	Oct. 11	+++	80.63	16.93	83.07	.774	67.58	4.18	71.76
7B	do	+	80.99	16.89	83.11	.763	67.12	3.79	70.91
7D	do	+++	80.60	18.76	81.24	.748	66.50	3.51	70.01
10A	Oct. 18	+++	79.78	16.62	83.38	.692	71.17	2.57	73.74
10B	do	+	80.62	14.71	85.29	.774	72.86	1.24	74.10
10D	do	+++	79.94	16.20	83.80	.773	70.54	1.99	72.53
11A	Oct. 24	+++	79.40	14.47	85.53	.704	73.54	1.80	75.34
11B	do	0	79.76	13.24	86.76	.692	73.07	1.78	74.85
11D	do	++	78.56	13.85	86.15	.676	72.06	2.61	74.57
11E	do	0	79.14	14.96	85.04	.527	71.19	3.07	74.26
11F	do	+	79.50	13.07	86.93	.634	71.66	1.71	73.37
11N [e]	do	++	79.02	15.59	84.41	.620	71.16	1.48	72.64
11M [e]	do	+	79.16	14.15	85.84	.648	72.84	1.82	74.66
11L [e]	do	+++	79.02	16.30	83.70	.543	71.21	2.53	73.74
11N [f]	do	++	78.25	14.02	85.98	.690	73.29	1.38	74.67
11M [f]	do	0	78.70	14.18	85.82	.610	74.27	1.69	75.96
11L [f]	do	+	78.36	14.28	85.72	.601	73.94	1.76	75.70

FUYU VARIETY [g]

2A	Sept. 15	(g)	84.40	22.71	77.29	0.674	66.07	0.90	66.97
2B	do	(g)	84.74	19.14	80.86	.721	70.51	0	70.51
2D	do	(g)	83.62	23.63	76.37	.678	71.31	0	71.31
4A	Sept. 22		84.00	19.81	80.19	.531	69.75	0	69.75
4B	do		84.45	16.66	83.34	.579	71.77	0	71.77
4D	do		83.78	19.48	80.52	.586	67.14	0	67.14
6A	Oct. 6		82.91	16.38	83.62	.497	71.62	3.98	75.60
6B	do		83.13	15.77	84.23	.474	72.44	3.91	76.35
6D	do		82.86	16.39	83.61	.496	71.76	3.85	75.61
9A	Oct. 13		81.78	16.30	83.70	.494	72.28	3.24	75.52
9B	do		81.98	13.60	86.40	.472	73.36	4.61	77.97
9D	do		82.09	15.25	83.75	.530	74.26	2.01	76.27
12A	Oct. 23		81.74	13.97	86.03	.438	75.36	2.52	77.88
12B	do		82.03	13.24	86.76	.473	75.91	3.12	79.03
12D	do		81.92	14.38	85.62	.498	75.78	2.05	77.83
13A	Oct. 27		79.16	12.09	87.91	.432	75.43	2.06	77.49
13B	do		79.74	10.37	89.63	.395	75.17	5.08	80.25
13D	do		79.16	11.52	88.48	.432	77.40	2.54	79.94
13E	do		79.46	10.52	89.48	.438	76.73	4.82	81.55
13F	do		79.60	12.11	87.89	.392	77.84	2.01	79.85
13N [e]	do		78.86	9.74	90.26	.426	79.42	2.74	82.16
13M [e]	do		79.51	10.59	89.41	.390	77.99	3.03	81.02
13L [e]	do		78.74	11.48	88.52	.376	79.12	2.07	81.19

[a] A and N, untreated, analyzed immediately after respiratory activity was determined; B and M, treated with ethylene; D and L, check, held under same conditions as B, but not treated; E, treated with ethylene for 1 week; F, check, held under same conditions as E.
[b] +=slightly astringent; ++=medium astringent; +++=very astringent.
[c] As anhydrous citric.
[d] As invert.
[e] Stored at room temperature for 1 week.
[f] Stored at room temperature for 2 weeks.
[g] Nonastringent variety.

The respiratory rate was thus ascertained for two lots, B and C, before treatment and also after treatment. Since, however, there was little difference between the respiratory rates of lots B and C, either before or after treatment, the average of these two was plotted. (Fig. 4.) In Table 2, B designates the average of the two lots B and C after treatment, and G is the average of the same lots before treatment.

TABLE 2.—*Respiration of Japanese persimmons before and after treatment with ethylene*

HACHIYA VARIETY

Sample No. [a]	Date of picking	Carbon dioxide	Oxygen	Ratio carbon dioxide oxygen at 0° C. 760 m. m.
	1927	*C. c. per kilo per hour*	*C. c. per kilo per hour*	
3A	Sept. 20	15.9	14.0	1.13
3D	do	25.3	22.8	1.11
3B	do	40.0	25.0	1.62
3G	do	15.0	14.6	1.04
Difference B−G		25.0	10.4	.58
5A	Sept. 27	14.7	14.0	1.05
5D	do	10.5	10.7	.98
5B	do	34.2	21.1	1.62
5G	do	15.5	15.6	1.00
Difference B−G		18.7	5.5	.62
7A	Oct. 11	19.9	20.3	.98
7D	do	24.8	24.4	1.22
7B	do	31.8	16.9	1.89
7G	do	17.4	17.0	1.02
Difference B−G		14.4	−.1	.87
10A	Oct. 18	17.2	15.5	1.11
10D	do	11.4	10.5	1.09
10B	do	28.7	20.1	1.43
10G	do	16.9	13.8	1.25
Difference B−G		11.8	6.3	.18
11A	Oct. 24	17.4	15.9	1.09
11D	do	18.9	13.4	1.36
11B	do	32.0	18.9	1.69
11G	do	17.5	16.1	1.09
Difference B−G		14.5	2.8	.60
11E	Oct. 24	23.4	6.5	3.60
11F	do	26.9	19.0	1.49
Difference E−F		−3.5	−12.5	2.11
11L [b]	Oct. 24	15.0	10.4	1.43
11M [b]	do	26.4	18.6	1.42
11N [b]	do	16.5	15.1	1.09
Difference M−N		9.9	3.5	.33
11L [c]	Oct. 24	21.2	14.7	1.44
11M [c]	do	25.2	12.5	2.01
11N [c]	do	19.1	16.7	1.14
Difference M−N		6.1	−4.2	.87

FUYU VARIETY

2A	Sept. 15	14.4	13.9	1.04
2D	do	11.1	13.1	.85
2B	do	29.9	24.9	1.20
2G	do	14.6	14.5	1.01
Difference B−G		15.3	10.4	.19
4A	Sept. 22	14.3	13.7	1.04
4D	do	10.9	10.5	1.04
4B	do	26.4	21.5	1.23
4G	do	14.1	13.1	1.08
Difference B−G		12.3	8.4	.15
6A	Oct. 6	13.6	14.1	.97
6D	do	11.7	10.1	1.16
6B	do	24.8	20.1	1.23
6G	do	13.5	13.6	1.00
Difference B−G		11.3	6.5	.23

[a] A, untreated; B and M, average of 2 lots, B and C, after treatment with ethylene; D and L, checks, held under same conditions as B but not treated; E, treated with ethylene for 1 week; F, check, held under same conditions as E but not treated; G and N, average of 2 lots, B and C, before treatment with ethylene.

[b] Stored at room temperature for 1 week.

[c] Stored at room temperature for 2 weeks.

TABLE 2.—*Respiration of Japanese persimmons before and after treatment with ethylene*—Continued

FUYU VARIETY—Continued

Sample No.	Date of picking	Carbon dioxide	Oxygen	Ratio carbon dioxide oxygen at 0° C. 760 m. m.
	1927	*C. c. per kilo per hour*	*C. c. per kilo per hour*	
9A	Oct. 13	13.3	13.4	0.99
9D	do	9.4	10.2	.92
9B	do	23.2	20.9	1.11
9G	do	13.4	13.3	1.00
Difference B−G		9.8	7.6	.11
12A	Oct. 23	13.0	12.9	1.01
12D	do	10.2	9.2	1.11
12B	do	25.9	19.5	1.33
12G	do	10.8	12.0	.90
Difference B−G	do	15.1	7.5	.43
13A	Oct. 27	14.9	15.1	.99
13D	do	12.2	10.4	1.17
13B	do	22.1	16.9	1.31
13G	do	13.6	12.8	1.06
Difference B−G		8.5	4.1	25.23
13E	Oct. 27	18.9	8.3	2.28
13F	do	11.9	10.1	1.18
Difference E−F		7.0	−1.8	1.10
13L [b]	Oct. 27	12.6	12.0	1.05
13M [b]	do	21.4	17.9	1.20
13N [b]	do	11.7	10.8	1.08
Difference M−N		9.7	7.1	.12

[b] Stored at room temperature for 1 week.

When the fruit was considered commercially mature, a large number of fruits were picked for storage tests as well as for maturity tests. The persimmons used for the storage tests were divided at the beginning of the storage period into lots of eight or nine fruits each designated as L, M, and N corresponding in treatment to lots D, B, and G mentioned above. These were stored at room temperature, some for one week and some for two weeks. (Tables 1 and 2.) Ethylene was applied for 46 to 52 hours at the end of the storage period. Only one lot was used to determine respiration after treatment.

Lots 11E and 13E were treated with ethylene for one week to determine the effect of ethylene over a longer period than that required for maturity tests. Lots 11F and 13F were held under the same conditions as untreated checks.

Temperatures during the period of treatment were recorded by means of thermographs. The maximum temperatures for all lots ranged from 20.2° to 27° C.; the average maximum was 21.7°. The range of minimum temperatures was from 14° to 19°, the average minimum being 16.6°.

ANALYTICAL METHODS

In determining color, the percentage of yellow surface as compared to green surface was estimated by eye for each fruit. Each analyst made independent observations, and from these the average for the whole sample was calculated.

The pressure was measured by means of the instrument devised by Magness and Taylor (*15*) for pears and apples, the plunger head being

seven-sixteenths of an inch in diameter. Two determinations on each of several unpeeled fruits were made, and the average was taken.

In preparing the samples for analysis, 10 fruits were cut from stem to stylar end, and half of each was discarded. The other halves, unpeeled, were then seeded, if necessary, and ground in a food chopper, first through a medium cutter and then through a nut-butter cutter. When this grinding proved unsatisfactory for the firm fruits they were grated on the fine teeth of a tin household grater.

The determinations of moisture and total solids were made on the same sample, by drying it to constant weight in vacuo at 70° C., according to official methods of the Association of Official Agricultural Chemists (*1, p. 210, No. 5*).

Insoluble solids were determined as follows: Twenty-five grams of the sample was boiled in 200 c. c. of distilled water for 30 minutes. After standing overnight, the material was filtered through closely woven muslin and thoroughly washed with water, and the residue was dried to constant weight at 100° C.

The quantity of soluble solids was determined by calculating the difference between total and insoluble solids.

Hydrogen-ion concentration, expressed as pH, was determined according to the LaMotte indicator method by matching the diluted juice (3 volumes of water to 1 of juice) against color standards of known value. Juice was obtained by squeezing the macerated pulp through several folds of cheesecloth.

For the determination of titratable acidity, 25 gm. of the pulp was boiled in 200 c. c. of distilled water for 30 minutes, filtered, washed, cooled, and titrated against 0.1562 normal sodium hydroxide (1 c. c. was equivalent to 0.01 gm. anhydrous citric acid), phenolphthalein being used as an indicator. Owing to the color in the Hachiya variety, it was necessary with this fruit to use a spot plate in determining the endpoint.

The following procedure was used in preparing the sample for the estimation of sugars: Ten grams of the pulp, into which approximately 0.5 gm. of calcium carbonate had been mixed, was boiled in 150 to 200 c. c. of 95 per cent alcohol for 30 minutes. This extraction was repeated twice with 80 per cent alcohol and once with distilled water. After evaporation of the alcohol, reducing sugars were determined according to official methods of the Association of Official Agricultural Chemists (*1, p. 190, No. 35*); the Munson and Walker method was used for reduction, and the cuprous oxide was titrated according to the volumetric permanganate method (*1, p. 192, No. 39*). Inversions were made by treatment with concentrated hydrochloric acid at room temperature for 24 hours or more. The quantity of sucrose was calculated by multiplying the difference between reducing sugars before and after inversions by the factor 0.95.

The respiratory activity was determined according to the method described by Johnstone (*9*) by which the samples were kept in an atmosphere which was approximately free from carbon dioxide and to which oxygen was supplied as it was removed, The quantity of oxygen absorbed by the water in the oxygen reserve cylinder was estimated to be 3 per cent of the water volume. Before they were immersed the respiratory chambers containing the fruit were brought approximately to the temperature of the water bath, kept at 25° C. by an electrically operated mercury thermoregulator.

From 100 to 200 c. c. of approximately normal sodium hydroxide served to absorb the carbon dioxide in each chamber. In most cases this quantity was sufficient to prevent any difficulty in titrating by the double-titration method, but occasionally it was necessary to use comparison standards for both end points.

RESULTS

The Hachiya appears to be physiologically the more active variety. The results from lots designated A (untreated) in Table 1 taken to follow maturity changes occurring with advance of the season clearly indicate this. Color (figs. 1 and 2), total sugars, and weight (fig. 3)

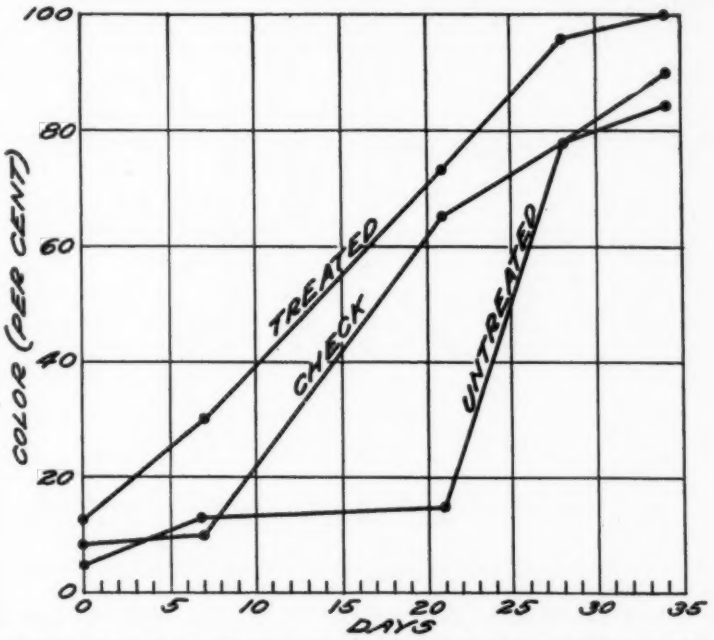

FIGURE 1.—Increase in color of Hachiya persimmons before and after treatment with ethylene during the sampling period

showed a greater increase and sucrose a greater decrease in the Hachiya. Moisture and insoluble solids seemed to decrease more in the Fuyu, but there was little difference in acidity changes. There was little change in either hardness or astringent taste, even though the last samples were picked after the fruits were fairly well colored.

The two varieties exhibited other differences. They differed in shape, and the Fuyu was smaller and colored more slowly than the Hachiya. The Fuyu possessed a larger quantity of total sugar, reducing sugar, soluble solids and moisture, but the Hachiya had the greater quantity of acid.

An interesting result was the sudden appearance of sucrose. With the exception of the untreated Fuyu in the first lot, no sucrose

appeared in either variety until the third week of sampling, October 6–11. After this the quantity in both varieties gradually decreased. Other workers seem to find sucrose present in the Japanese persimmon (*8, 16, 18*). Komatsu and Ishimasa (*12*) report an increase of sucrose in one variety of Japanese persimmon as it ripened.

The greater respiratory activity of the Hachiya revealed by the data which have been reduced to standard conditions of 760 mm. and 0° C. is shown in Figure 4 in the upper set of curves, which were plotted from triplicate lots. The quantities of oxygen intake and carbon dioxide output were consistently larger in the Hachiya. Possibly a little more oxygen, relative to carbon dioxide output, was taken in by the Fuyu, as a comparison of the respiratory ratios suggests. The peaks of the curves for the Hachiya were reached October

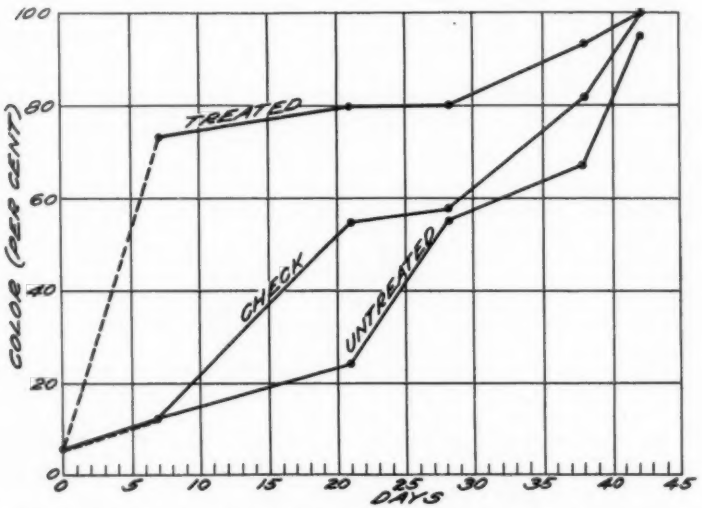

FIGURE 2.—Increase in color of Fuyu persimmons before and after treatment with ethylene during the sampling period

6–11, but the curves for the Fuyu show little rise or fall. In considering the effect of ethylene, this physiological difference should be noted.

In order to determine the effect of ethylene, the check and treated lots were compared. Student's method (*13, 14*) of calculating odds appeared to find particular application here, and accordingly was used as an aid in determining the significance of the results. When the curves for the treated and check lots were practically the same over part of the curve but diverged for the remainder, the odds were calculated in two ways—for the whole period, and for the period of divergence only. In no case did the odds reach 30 to 1, below which differences are not regarded as significant by either method of calculation.

The most pronounced effect of ethylene treatment was to cause a rapid softening (fig. 5), a change readily produced at any of the stages of maturity studied. The data for all treated lots were alike in that they lay entirely outside the range of the checks. It is probable that the after-treatment data record only the toughness of the peel.

Next in importance to the softening effect of ethylene was its effect on the color, which was considerably increased in the Hachiya. In

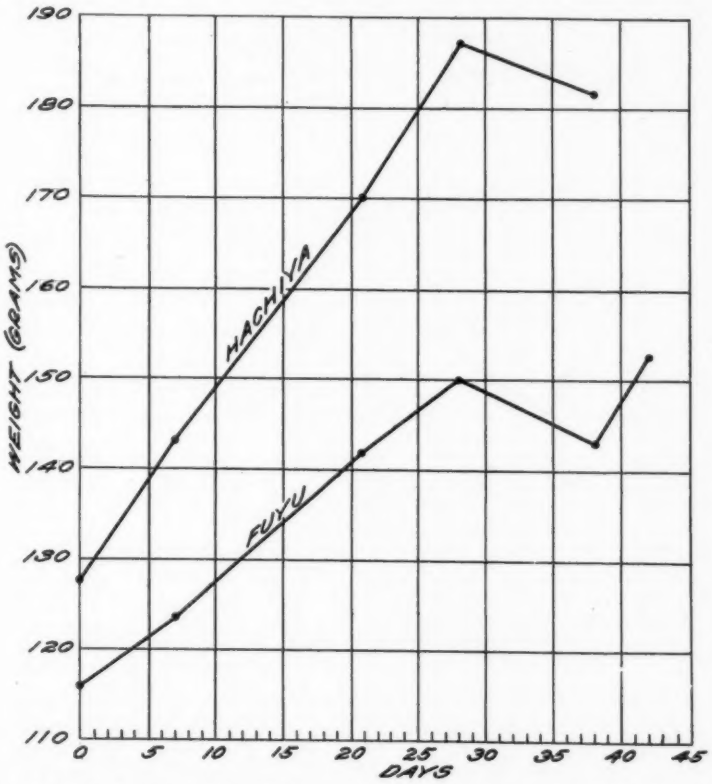

FIGURE 3.—Increase in weight of Hachiya and Fuyu persimmons during the sampling period

the Fuyu the odds for increased color are rather low, 34 to 1, but it is possible that a longer treatment is necessary for this less active fruit, and the method of estimating color should also be taken into consideration.

Significant changes occurred in three other constituents as a result of the treatment. In the Fuyu the insoluble solids decreased and simultaneously the soluble solids increased (figs. 6 and 7), but it is uncertain whether or not the same changes took place in the Hachiya. The third constituent affected was moisture (fig. 6) in the Hachiya,

and though the differences were rather small, 0.2 to 1 per cent, they were consistent, yielding high odds. There is not enough difference between the curves for the Fuyu to indicate with certainty the same increase in moisture.

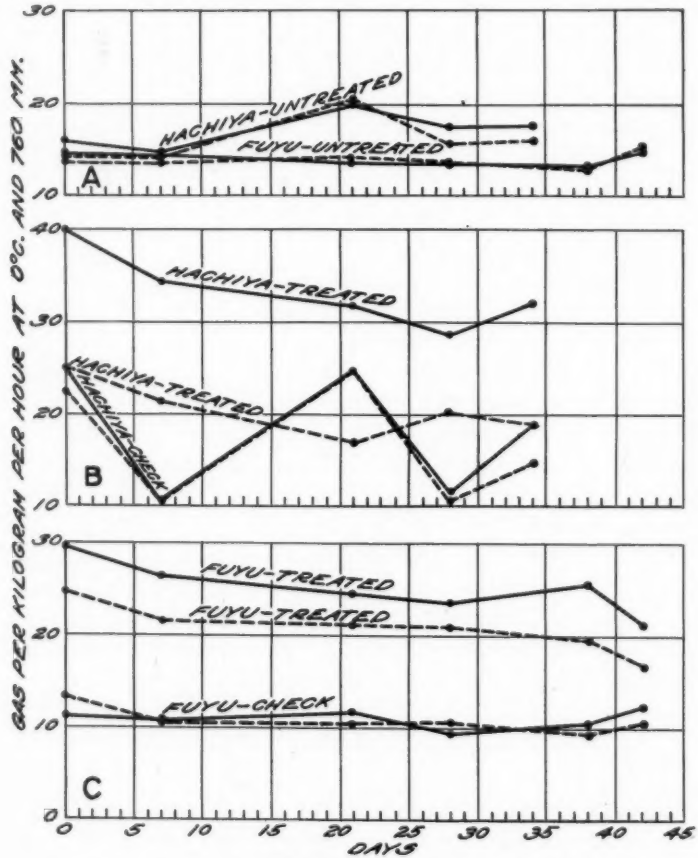

FIGURE 4.—Changes in respiratory activity of Hachiya and Fuyu persimmons: A, with advance of season; B and C, after treatment with ethylene. Solid lines represent carbon dioxide given off by fruit; broken lines the oxygen taken in

The odds for increase of sucrose, reducing sugar, or total sugars after treatment are too low to be significant for either variety. (Table 3 and fig. 8.) In fact, the odds are not such as to indicate definitely the effect of ethylene on the sugars. These results, however, do suggest the need for further study of the effect of ethylene on this constituent.

TABLE 3.—*Effect of ethylene treatment upon sugar content of Japanese persimmons (dry-weight basis)*

REDUCING SUGARS

Date of picking	Hachiya variety			Fuyu variety		
	Check lot	Treated lot	Difference	Check lot	Treated lot	Difference
1927	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Sept. 15, 20	55.56	56.59	+1.03	71.31	70.51	−0.80
Sept. 22, 27	64.10	63.76	−.34	67.14	71.77	+4.63
Oct. 6, 11	66.50	67.12	+.62	71.76	72.44	+.68
Oct. 13, 18	70.54	72.86	+2.32	74.26	73.36	−.90
Oct. 23, 24	72.06	73.07	+1.01	75.78	75.91	+.13
Oct. 27				77.40	75.17	−2.23

SUCROSE

Oct. 6, 11	3.51	3.79	+.28	3.85	3.91	+.06
Oct. 13, 18	1.99	1.24	−.75	2.01	4.61	+2.60
Oct. 23, 24	2.61	1.78	−.83	2.05	3.12	+1.07
Oct. 27				2.54	5.08	+2.54

TOTAL SUGARS

Sept. 15, 20	55.56	56.59	+1.03	71.31	70.51	−.80
Sept. 22, 27	64.10	63.76	−.34	67.14	71.77	+4.63
Oct. 6, 11	70.00	70.91	+.91	75.61	76.35	+.74
Oct. 13, 18	72.53	74.10	+1.57	76.27	77.97	+1.70
Oct. 23, 24	74.67	74.85	+.18	77.82	79.02	+1.20
Oct. 27				79.94	80.26	+.32

Ethylene affected the respiratory activity of both varieties of persimmons in a marked degree. (Table 2.) The oxygen intake and carbon dioxide output, especially the latter, were greatly stimulated, as may be seen in the two lower sets of curves in Figure 4. Two exceptions occurred, in lots 3D and 7D, but the increase in these checks may have been due to accidental exposure of the fruit to ethylene in airing the desiccators. In the more active Hachiya, the maximum production of carbon dioxide (fig. 4) showed an increase of 167 per cent. Oxygen intake did not keep pace with carbon dioxide evolved, which resulted in a tendency for the respiratory ratio, $\frac{CO_2}{O_2}$, to increase in the later stages of ripening, especially in the Hachiya. That longer periods of treatment would have further increased this ratio is indicated by the results of treating lots 11E and 13E for 168 hours. (Table 2.)

The stimulative effect of ethylene depends upon the stage of maturity of the fruit to which it is applied, decreasing as the fruit becomes more mature. This decrease appears to bear no direct relation to the respiratory changes taking place during maturation.

An attempt made to follow the changes in astringency accurately by the official tannin method (*1, p. 340, No. 18*) for tea, was so unsatisfactory that only the results secured roughly by tasting are given. These show that the astringent taste decreased after treatment. (Table 1.) The total acidity was not greatly affected by ethylene, as may be seen in Figure 8. The pH values of water extracts of the fruits were determined, but they varied so little that the data are not given.

Unfortunately little attention could be given to storage experiments, but the results indicate that there was little deviation from the trend of changes taking place previous to storage. The decrease in astringent taste, however, was evident in the stored fruit.

DISCUSSION

According to the data for the two varieties studied, the chief effect of ethylene is on hardness, color development, and respiration, particularly the first. This softening effect is apparently not related to the stage of maturity at which ethylene is applied, quite in contrast to the effect on color and respiration. The fact that the

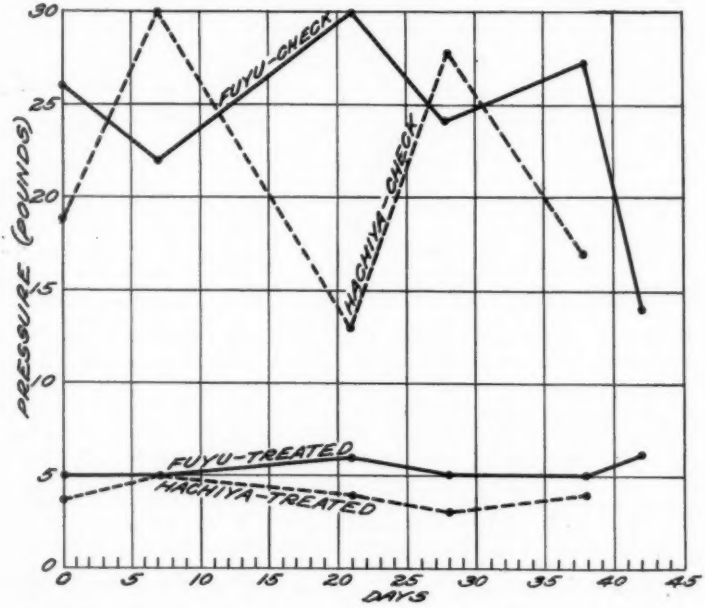

FIGURE 5.—Changes in hardness of Fuyu and Hachiya persimmons before and after treatment with ethylene, during the sampling period. Pressure is given in pounds necessary to penetrate skin with a 7/16-inch head

persimmon does not normally become softer with the advance of the season probably renders the pressure test of little value in determining maturity.

Associated with the softening there is an increase of soluble solids and decrease of insoluble solids as a result of treatment with ethylene. The change is unquestionable for the Fuyu, and though the odds are so low as to be doubtful for the Hachiya, nevertheless both the direction and shape of the curves suggest that the same change may have taken place. The increase of soluble solids would not be so great in the more active Hachiya if the soluble material was removed by respiration.

These data do not indicate that increase of soluble solids is due to increase of total acids and total sugars. Rather it seems more likely to be caused by changes in pectic substances or hemicelluloses, but unfortunately lack of time prevented the determination of these substances.

Harvey (*10*) reports an increase in alcohol-ether-soluble material in the epicotyl of the sweet pea after ethylene treatment. High odds for both increased soluble solids and osmotic pressure were revealed

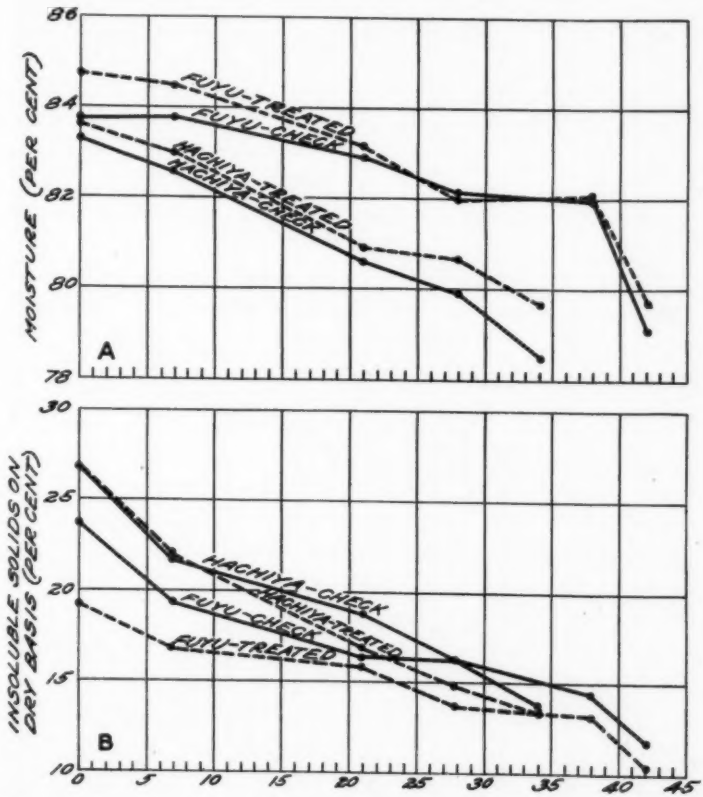

FIGURE 6.—Changes in moisture (A) and in insoluble solids (B) of Hachiya and Fuyu persimmons before and after treatment with ethylene during sampling period

when his data had been calculated for odds, but ethylene decreased the respiration rate. It seems rather difficult, however, to compare two tissues so diverse in nature and especially so diverse in their response to environmental conditions.

Ethylene brought about an increase in color even in the very immature fruit, although such fruit later became discolored. Experience has shown that longer treatment of immature fruit does not produce a well-colored product, nor will fruit picked too green and

held in storage for some time color afterward if treated. Although the estimation of color is rather difficult and color as a standard of maturity is hard to apply, it is clear that better results are obtained in artificial coloring of persimmons if they are picked at the proper stage of maturity.

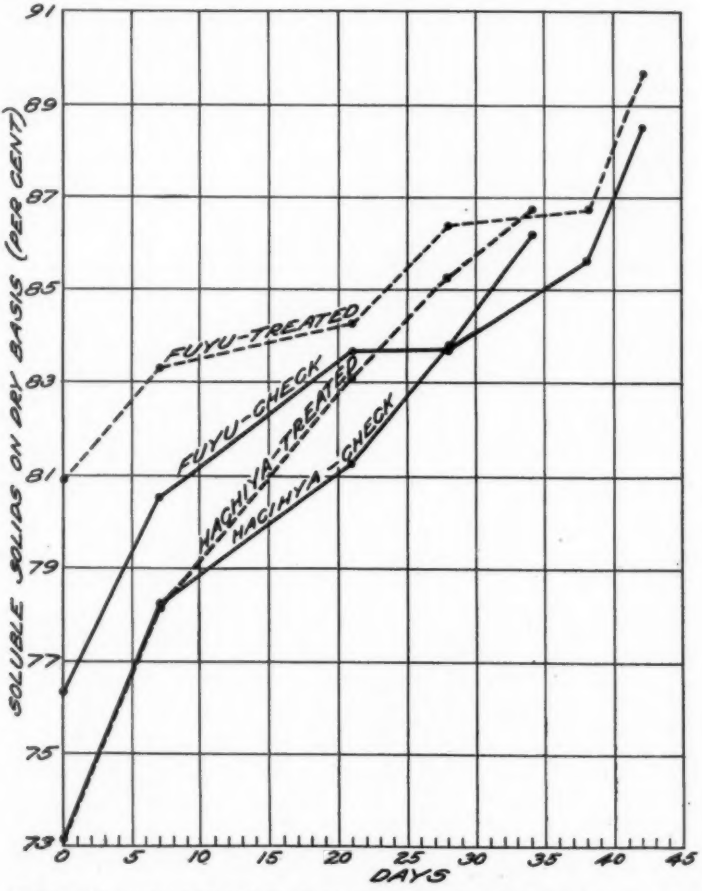

FIGURE 7.—Changes in soluble solids (dry basis) of Hachiya and Fuyu persimmons before and after treatment with ethylene during the sampling period

The data here reported indicate that fruit 80 per cent colored has more than 95 per cent of the total sugars and nearly the maximum weight of the ripe fruit. These data, however, are the results of but one season's study, and might not be obtained another season, as seasonal fluctuations in behavior are known to occur in other fruits, as for example the pomegranate (*4*). Total sugars in these persim-

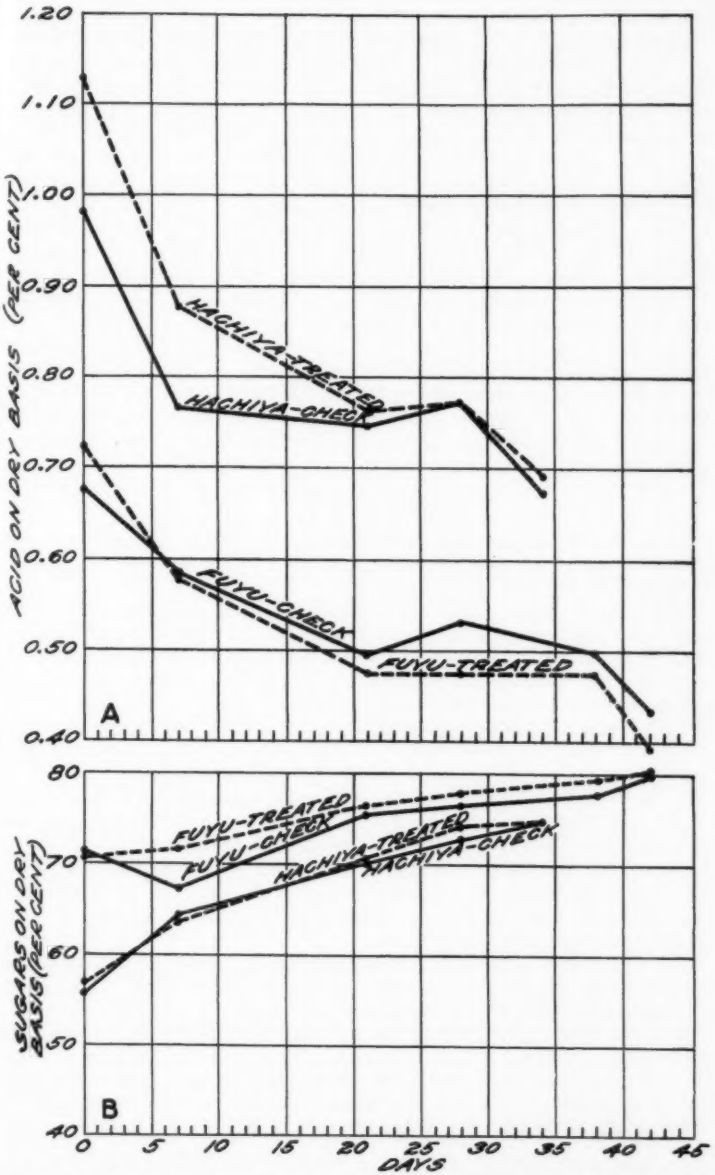

FIGURE 8.—Changes in acid (A) and in total sugars (B) (dry basis) of Hachiya and Fuyu persimmons before and after treatment with ethylene during the sampling

mons reached a maximum rather early, but this might not occur another season.

The importance of the greater physiological activity of the Hachiya revealed by these studies should be noted. If increased respiration explains the increased moisture of the treated Hachiya, then the lesser physiological activity of the Fuyu may explain why the odds for increased moisture and color development are so low for this variety. A longer exposure may be necessary to obtain the same degree of color and moisture increase as for the Hachiya. Age and climate may also complicate these results.

Although the softening effect, the most striking result of ethylene treatment of persimmons, may limit the use of ethylene to treatment of fruits intended for local distribution, the enhancement of color and decrease in astringent taste, making a more uniform, marketable, and palatable product, seem to be of practical advantage.

The results of these studies do not indicate any one easily explained cause of the effect of ethylene on persimmons. Since the respiratory rate is disturbed, the effect seems to be on the general metabolism with subsequent changes in chemical constituents. Further investigation of these softening and color changes is of great importance to the fruit and vegetable industry.

SUMMARY

Attention is directed to the desirable qualities of Japanese persimmons, their increased production, and the practical advantages of treating them with ethylene.

A study of some physical, chemical, and respiratory changes occurring during the growth and ripening of a nonastringent and an astringent variety of Japanese persimmon, and of the effect of a low concentration of ethylene, applied at intervals during maturation, on the changes mentioned, is reported.

The period of study extended from September 15, when the fruit was almost entirely green but of nearly full size, to October 27, 1927, when it was considered commercially mature.

It was found that physiologically the Hachiya is more active than the Fuyu. Color, total sugars, reducing sugars, and weight showed a greater increase and sucrose a greater decrease in the Hachiya. Moisture and insoluble solids decrease, however, was greater in the Fuyu. The respiratory activity of the two varieties also differed, both in amount of gaseous exchange and in fluctuations in activity with the season's advance.

The Fuyu has a greater quantity of sugar, soluble solids, and moisture, but the Hachiya appears slightly more acid. The Fuyu develops color more slowly than the Hachiya.

Ethylene treatment stimulated softening, color development, and respiratory activity in both varieties. Astringent taste decreased and moisture increased in the Hachiya, and in the Fuyu insoluble solids decreased simultaneously with increase of soluble solids.

The stimulative effect of ethylene on respiration declined, and the respiratory quotient $\frac{CO_2}{O_2}$ showed a tendency to increase as the fruit ripened, especially in the last stages and in the stored fruit.

Results of storing the less active mature persimmons, untreated, at room temperatures indicate for most constituents, except astringent taste in the Hachiya, which decreased rapidly, a continuance of the changes already begun on the tree.

Ethylene seems to act on the varieties of persimmons that were studied through its effect on the general metabolism.

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THE MICROFLORA OF A RICH SULPHATE-CONTAINING SOIL[1]

By J. Dudley Greaves
Utah Agricultural Experiment Station

CHARACTER OF THE SOIL

Some alkali soils are extremely rich in sulphates but contain only small quantities of other salts. Such a soil was used in this study. Areas within the district from which the soil was obtained were so high in soluble salts, mainly sodium sulphate, that only a few alkali-tolerant plants grew upon it. However, the surrounding soil, which contains only a low salt content, is highly productive. The samples were taken from the "alkali spots." Ten-kilogram portions were leached for two years.[2] Ninety liters of water passed through the soil, which, in addition to small quantities of other salts, removed 67.8 gm. of sodium sulphate and 12.7 gm. of sodium chloride.

After the soil was leached one crop of crimson clover and two crops of barley were grown on it. However, the yields in all cases were only slightly greater on the leached than on the unleached soil. The soil was sampled after each crop and its ammonifying, nitrifying, and nitrogen-fixing powers determined. The leaching of the soil greatly increased the number and activity of the microorganisms within it.[3] Its nitrogen-fixing powers were very active, for when kept in pots in the greenhouse with optimum moisture and nearly optimum temperature for a period of three years it gained nitrogen at the rate 1,142 pounds per acre-foot of soil.[4]

METHOD OF STUDY

The soil for this study was taken from the pots after the second crop of barley had been harvested. It was carefully ground and mixed under conditions such that foreign organisms did not enter. It was then plated on synthetic Ashby and nutrient agar in dilutions of 1 to 20,000 and 1 to 200,000. The average results were: On nutrient agar, 5,600,000 colonies; on synthetic agar, 3,878,000 colonies; and on Ashby agar, 3,855,000 colonies. Apparently the microflora of this soil more nearly approach those of the synthetic alkali soil[5] than they do those of the chloride-containing soil, for in the sulphate and synthetic alkali leached soil the counts on the Ashby agar were low, whereas the chloride-leached soil yielded high counts on the Ashby agar. Both soils were of the same geological origin and were quite similar in physical and chemical properties, except that the soil considered in this paper contained mainly sulphates before leaching,

[1] Received for publication Sept. 19, 1930; issued February, 1931. Contribution from the Department of Chemistry and Bacteriology, Utah Agricultural Experiment Station.

[2] Greaves, J. E. The microflora and productivity of leached and nonleached alkali soils. Soil Sci. 23: 271–302. 1927.

[3] Greaves, J. E. Op. cit. (See footnote 2.)

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whereas the synthetic alkali soil contained before leaching equal quantities of sodium sulphate, sodium chloride, and sodium carbonate.

The different colony types appearing on the Ashby agar were obtained in pure cultures and studied morphologically and physiologically, as described in preceding papers.[6] Twenty-one organisms were studied in detail (Table 1), but only the more active ammonifiers and nitrogen fixers (16 in number) are discussed.

TABLE 1.—*Summary of chief physiological and morphological properties of microorganisms* [a] *obtained from an alkali soil rich in sodium sulphate*

Culture No.	Nitrate reduction	Gelatin liquefaction	Starch hydrolysis	Milk peptonization	Indol production	Litmus-milk reaction	Action on glucose	Action on lactose	Action on sucrose	Shape [b]	Motility	Gram reaction	Chromogenesis	Spore formation	Ammonification	Nitrogen fixation
201B	−	+	+	+	+	−	+	0	+	F	−	+	−	+	2.6	2.8
202A	−	+	+	+	−	−	0	0	0	C	−	−	−	−	2.0	0
202B	+	+	+	+	+	−	+	0	0	B	−	−	+	−	2.3	4.2
202C	−	+	+	+	+	0	+	0	+	B	−	+	−	−	0	1.4
203A	−	+	+	+	−	−	+	0	0	F	−	+	−	−	6.1	1.4
208A	+	+	+	+	+	−	+	0	+	B	+	+	+	+	9.7	2.8
210B	−	+	+	−	+	0	+	0	0	B	+	+	+	−	9.9	0
210D	−	+	+	−	−	−	+	0	0	B	+	+	+	−	7.0	0
212C	−	+	+	−	+	0	0	+	0	B	+	+	−	+	0	2.8
212E	−	−	−	+	−	0	0	0	0	F	−	+	+	+	.3	1.4
214	−	+	−	−	+	+	+	+	+	B	+	+	+	−	1.9	1.4
217	+	+	+	−	−	−	0	0	0	B	−	+	−	−	0	2.8
219A	+	−	−	−	−	+	+	0	0	C	−	+	+	−	1.2	2.8
219B	−	+	+	+	+	−	0	0	0	F	−	+	+	+	14.6	0
221B	+	+	+	+	+	−	+	0	0	B	+	+	+	+	9.9	2.8
223	+	+	+	+	+	−	+	0	+	B	+	+	+	+	25.9	0
225A	−	+	+	+	+	−	+	0	+	F	−	+	+	+	8.8	4.5
225C	+	+	+	−	−	+	+	+	0	B	+	+	+	+	7.1	2.8
226	−	+	+	−	+	+	+	+	0	B	−	+	−	+	8.5	1.4
227A	+	+	+	+	+	+	+	0	0	F	−	+	+	+	1.5	7.0
228B	+	+	+	−	−	−	0	0	0	B	−	+	+	−	4.8	0

[a] All given here are aerobic.
[b] F=filamentous, C=cocci, B=bacilli.

CULTURAL CHARACTERS OF THE ORGANISMS

FUNGI

CULTURE 201B.—Mycelium septate with slight branching. Conidiophore with conical masses as well as chlamydospores. Abundant growth on ordinary laboratory media. Liquefies gelatin. Rapidly peptonizes milk, with alkaline reaction. Does not reduce nitrates, but produces gas on potassium nitrate broth. No soluble pigment. Produces indol; hydrolyzes starch. Produces acid on glucose and sucrose without the production of gas. Aerobic. Produces 2.6 mgm. of ammonia in peptone and fixes 2.8 mgm. of nitrogen in soil containing mannite.

CULTURE 203A.—Mold. Mycelium septate, with branching sporangia and chlamydospores. Rapid growth on gelatin with liquefaction and with browning of media. Abundant growth on agar and in broth. Abundant white growth with bluish-green borders on potato. Does not produce indol nor reduce nitrates. Produces acid on glucose without the production of gas. Hydrolyzes starch. Aerobic. Produces 6.1 mgm. of ammonia in peptone and fixes 1.4 mgm. of nitrogen in soil.

CULTURE 219B.—Mold. Mycelium septate and branched. Serial mycelium and conidia. Abundant bluish-green growth on agar. Rapid liquefying growth on gelatin with browning of the media. Abundant bluish-green filamentous growth on potato. Peptonization of milk, with alkaline reaction. Produces indol and hydrolyzes starch. Does not produce acid on carbohydrate media. Aerobic. Produces 14.6 mgm. of ammonia in peptone but does not fix nitrogen.

[6] GREAVES, J. D. THE MICROFLORA OF LEACHED ALKALI SOILS: SYNTHETIC ALKALI SOIL. Soil Sci. 28: 341-356. 1929.
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CULTURE 225A.—Mold. Mycelium septate and branched. Sporangia formed as in Mucor. Aerial mycelium nonseptate. Abundant blue-green growth on gelatin and potato. Gelatin liquefied. Moderate growth on agar and in broth. Peptonizes milk, with alkaline reaction. Produces indol but does not reduce nitrates. Produces acid on glucose and sucrose but not on lactose. Hydrolyzes starch. Aerobic. Produces 8.8 mgm. of ammonia in peptone and fixes 4.5 mgm. of nitrogen in soil.

CULTURE 227A.—Actinomyces. Mycelium straight with very little branching. Rapid and abundant growth on agar, broth, and gelatin. Liquifies gelatin. Moderate white growth with darkening on potato. Peptonizes milk with the production of acid. Produces indol, reduces nitrates, and hydrolyzes starch. Produces acid on glucose and actively decomposes cellulose. Aerobic. Produces small quantities of ammonia in peptone and fixes 7.0 mgm. of nitrogen in soil.

BACTERIA

CULTURE 202B.—Gram-negative, nonmotile, nonspore-forming; rods 0.5 to 0.7 by 1.2 to 1.9 μ, occurring singly and in clusters. Grows rapidly on agar. Grows slowly on gelatin, with liquefaction. Makes abundant tan-colored growth on potato. Slowly peptonizes litmus milk, with alkaline reaction. Reduces litmus. Produces indol. Reduces nitrates, hydrolyzes starch, and produces acid in glucose media. Aerobic. Produces 2.3 mgm. of ammonia in peptone and fixes 4.2 mgm. of nitrogen in soil.

CULTURE 208A.—Gram-positive; rods, 0.7 to 0.8 by 4.0 to 10.0 μ, occurring singly and in pairs. Motile by means of peritrichous flagella. Produces central ellipsoidal spores 0.8 by 1.5 μ. Grows rapidly on gelatin, with liquefaction. Makes abundant greenish-brown growth on agar and good growth in broth and on potato. Peptonizes milk, with alkaline reaction. Produces indol, reduces nitrates, produces acid on glucose and sucrose. Hydrolyzes starch and liberates hydrogen sulphide from peptone. Facultative aerobe. Produces 7 mgm. of ammonia from urea and 9.7 mgm. from peptone. Fixes 2.8 mgm. of nitrogen in soil.

CULTURE 210B.—Gram-positive, nonspore-forming; rods 0.7 to 0.8 by 3.3 to 4.5 μ, occurring singly, in pairs, and in short chains. Motile by means of peritrichous flagella. Makes abundant growth on all ordinary laboratory media. Rapidly liquefies gelatin. Causes no change in milk. Produces indol but does not reduce nitrates; produces acid on glucose, and hydrolyzes starch. Aerobic. Produces 9.9 mgm. of ammonia in peptone but does not fix nitrogen.

CULTURE 210D.—Gram-positive, nonspore-forming; rods 0.8 to 1.2 by 2.9 to 7.5 μ, occurring singly, in pairs, and in chains. Motile by means of peritrichous flagella. Makes abundant growth on agar, gelatin, and broth, but no growth on potato. Rapidly liquefies gelatin and turns milk alkaline. Does not form indol, nor reduce nitrates. Produces acid on glucose and hydrolyzes starch. Aerobic. Produces 7 mgm. of ammonia in peptone but does not fix nitrogen.

CULTURE 212C.—Gram-positive; rods 0.8 to 0.9 by 4.0 to 6.1 μ, occurring singly and in chains. Form central ellipsoidal spores 1.1 by 1.9 μ. Motile by means of peritrichous flagella. Grows rapidly on agar and slowly with liquefaction on gelatin. Produces no change in litmus milk. Produces indol, does not reduce nitrates, produces acid in lactose, and hydrolyzes starch. Facultatively aerobic. Does not produce ammonia in peptone but fixes 2.8 mgm. of nitrogen in soil.

CULTURE 217.—Gram-positive, nonmotile, nonspore-forming; rods 0.45 to 0.6 by 1.2 to 2.4 μ, occurring singly and in clusters. Grows rapidly on agar and broth. Grows slowly with liquefaction on gelatin. Reduces litmus milk with alkaline reaction. Does not produce indol; does reduce nitrates; does not produce acid on carbohydrate media. Hydrolyzes starch. Aerobic. Fixes 2.8 mgm. of nitrogen in soil.

CULTURE 219A.—Gram-positive, nonmotile, nonspore-forming; micrococci 0.6 to 0.9 μ in diameter. Makes abundant growth on agar, slow growth on gelatin without liquefaction, and no growth on potato. Does not produce indol but reduces nitrates. Does not hydrolyze starch. Aerobic. Produces 1.2 mgm. of ammonia on peptone and fixes 2.8 mgm. of nitrogen in soil.

CULTURE 221B.—Gram-positive, motile; rods 0.8 to 0.9 by 3.6 to 5.5 μ, occurring singly, in pairs, and in short chains. Central ellipsoidal spores 0.8 by 1.8 to 2.2 μ. Makes abundant growth on ordinary laboratory media. Liquefies gelatin. Peptonizes milk, with an alkaline reaction. Produces indol; reduces nitrates; produces acid on glucose. Hydrolyzes starch. Aerobic. Produces 9.9 mgm. of ammonia in peptone and fixes 2.8 mgm. of nitrogen in soil.

CULTURE 223.—Gram-positive; rods 0.8 to 1.1 by 2.8 to 8.7 μ. Motile by means of peritrichous flagella. Forms central spores 1.0 to 1.1 by 1.8 to 2.1 μ. Makes abundant growth on all ordinary laboratory media. Liquefies gelatin, darkens potato. Slowly peptonizes litmus milk with alkaline reaction and the reduction of the litmus. Produces indol, reduces nitrates, produces acid on glucose and sucrose, and hydrolyzes starch. Produces hydrogen sulphide. Aerobic. Produces 25.9 mgm. of ammonia in peptone but does not fix nitrogen.

CULTURE 225C.—Gram-positive; rods 0.8 to 1.8 by 4.5 to 13.7 μ, occurring in chains. Motile by means of peritrichous flagella. Forms central ellipsoidal spores. Makes rapid growth on agar, gelatin, and broth. Liquefies gelatin. Coagulates milk, with acid reaction. Does not produce indol, reduces nitrates, and produces acid in glucose and lactose. Hydrolyzes starch. Aerobic. Produces 7.1 mgm. of ammonia in peptone and fixes 2.8 mgm. of nitrogen in soil.

CULTURE 226.—Gram-positive, nonmotile; rods 0.8 to 0.9 by 2.7 to 3.2 μ, occurring singly. Forms central spores 1.1 to 1.6 by 2.1 to 2.8 μ. Makes rapid and abundant growth on agar and broth and slow growth on gelatin, with liquefaction. Produces acid in milk. Forms indol, hydrolyzes starch, but does not reduce nitrates. Aerobic. Produces 8.5 mgm. of ammonia in peptone and fixes small quantities of nitrogen in soil.

SUMMARY

This study shows the physiological and morphological properties of 21 microorganisms obtained from a natural-occurring alkali soil which had been partially reclaimed by leaching. Of the 21, 6 were fungi and 15 were bacteria; 13 of the bacteria were bacillus forms and 2 were coccus forms. Eight of the bacilli were motile, 6 formed spores, and 19 of the organisms were Gram-positive. Nineteen liquefied gelatin, 18 hydrolyzed starch, 12 peptonized milk, 9 reduced nitrates, 13 produced indol; 15 fermented glucose, 4 lactose, and 6 sucrose.

Eighteen produced ammonia when grown in peptone. The quantity of ammonia produced in four days ranged from 0.3 mgm. to 26 mgm. Ten of the organisms produced over 5 mgm. of ammonia. Fifteen of the organisms fixed nitrogen when grown in soil to which 1 per cent of mannite had been added. The quantity fixed varied from 1.4 to 7 mgm. in 100 gm. of soil. Three fixed over 4 mgm., and these are probably the organisms, or at least among the organisms, which are causing this soil to gain nitrogen. Two of the organisms decomposed cellulose and one liberated ammonia from urea.

U. S. GOVERNMENT PRINTING OFFICE: 1931